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MALARIA STUDIES IN GREECE

A SURVEY OF MALARIA MORBIDITY IN A REGION OF EAST MACEDONIA*

BY

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(*Received for publication* 13 *June*, 1935)

The purpose of this survey was to obtain data on the amount of actual illness due to malaria in a region of East Macedonia. Nineteen villages, representing various degrees of malarial endemicity, were visited. All are situated between the Strumon and Nestos Rivers, the great majority of them in or immediately adjoining the Plains of Philippi and Chrysoupolis. The information was obtained by means of house-to-house visits by two physicians (H.P.C. and A.M.), both familiar with clinical malaria. The investigators were not content with mere samples of village houses : nearly every house of a village was visited at least once during the survey.

The data obtained include clinical signs and symptoms, clinical history, and blood parasite and spleen findings. It is obvious that enlargement of the spleen or the presence of parasites in the blood does not alone indicate illness. In some of our villages 60 per cent. of children quite able to attend school showed parasites in the blood, and 80 per cent. or more showed enlarged spleens. Enlargement of the spleen, especially, may persist long after clinical attacks have disappeared or become rare. Blood and spleen data were used largely as confirmatory evidence of the clinical condition. Blood findings, in this respect, are probably more valuable than spleen findings, and we have introduced the parasite rates in the report of results given in Table I.

The survey was made during the period of maximum malaria in this region, August 13th to November 26th. All visits but one (Lithotopos) were made before October 3rd. The year 1934 was an ' off ' year for malaria in Greece generally, but it certainly was not so in all the region surveyed.

The villages surveyed are grouped according to the amount of epidemic or endemic malaria, and to the probable degree of malaria transmission, both during 1934. The classification is based on our routine blood parasite and spleen surveys, on the sporozoite rate of large numbers of anophelines dissected, and on an October blood parasite survey of infants 11 months old or younger. The last was of especial value in determining the amount of transmission. In one whole region, Group D, the infant parasite rate, 1934, was almost nil ;

* The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of the Rockefeller Foundation.

in another, Group B, it was over 25 per cent. Group C is intermediate between B and D in nearly all respects. In the first group, A, which contains but one village, the evidence for an epidemic condition was good. The parasite survey of school-children showed nearly 100 per cent. positive (a sharp increase over the rate of the preceding spring), and a relatively high percentage of the population was found ill at the time of our survey, November 26th. A list of villages follows :

VILLAGE GROUPS

A. *Epidemic*—Lithotopos.

B. *Highly endemic*—Transmission of malaria present in 1934. The autumn parasite rates, 1934, of village children, mostly of school age, are as follows :

		Per cent.
1.	Kineze (Proastion)	55
2.	Peristereon	59
3.	Haidefto	60
4.	Piyes	63
5.	Monastiraki	63
6.	Nees Karies	60

C. *Endemic*—A decline of 13-26 per cent. in the parasite rate since our first examination in 1932 or 1933. Transmission continued during 1934, but apparently in diminishing degree. The blood parasite rate of the autumn of 1934 is shown below :

		Per cent.
1.	Chrysochori	41
2.	Ayiasma	44
3.	Kalamon	33

D. *Diminished endemic*—The blood parasite rate diminished from a range of 55–60 per cent. in the spring of 1932 to a range of 3–20 per cent. in the autumn of 1934. The autumn index of 1934 is shown in the list below. Transmission was nearly, if not quite, nil during 1934. Anophelism has greatly diminished on account of meteorological conditions. The cases of malaria that were found were probably all due to relapses.

		Per cent.
1.	Amissiana	3
2.	Polystylos	10
3.	Mavrolefki..	16
4.	Lefki A and B	7
5.	Eratinon	20
6.	Amygdaleon	6
7.	Datos	20
8.	Kara Orman	10

E. *Mountain villages*—The first gave zero in a survey of the indigenous population, July, 1932. The second showed a decline from 21 per cent. in 1932 to 3 per cent. in 1934. Acute malaria at present is practically nil, except for a few cases which gave a clear history of residence in the plains. These are omitted in our summaries. The group serves fairly well as a control.

1. Macryhori
2. Krinides

The population of the different groups is shown in Table I.

In Table I we have summarized the results of our surveys of these village groups, arranged according to the degree of epidemic or endemic malaria. We have also classified cases according to the amount of evidence for the presence of clinical malaria. In the first column, under ' Completely incapacitated,' are included persons either sick in bed or so ill that they ought to be in bed. Under ' Partially incapacitated ' are those able to move about the house or yard, but definitely indisposed. The character of the individual as well as the severity of the disease, of course, determines whether he takes to bed or not when ill. The first column includes a higher percentage than does the second of those who showed more definite signs of malaria, such as rigors, temperature and recent history of intermittent fever. In the third column are those who showed no signs of acute disease but gave a history of malaria within seven days. In each village and clinical group both total cases and persons parasite-positive are classified according to number and incidence in the population. Some of the cases had been treated by a physician, or the patients had taken quinine on their own initiative ; so that negative blood does not exclude malaria. In our blood examinations, we estimated the degree of infestation of the blood in terms of numbers of parasites per thick-film field. After analyzing the data with respect to the relation of degree of infestation to clinical symptoms, we did not think this factor of sufficient significance to include in the tables of results.

In this paper, patients ' completely incapacitated ' or ' partially incapacitated ' will be referred to as ' clinical ' cases, and those with history alone as ' history ' cases. We use these labels merely for convenience in description, making no pretence of exact definitions.

Comment on Table I. In each of the two clinical columns and in the history column, the number of cases per 100 of population declines with the decrease of malarial epidemicity or endemicity. This is what we should expect if our cases, or a large proportion of them, are really due to malaria. On the other hand, the parasite rate also declines with endemicity, a tendency we should not expect, for there is no reason why sick people in less malarious villages should take more quinine than those in more malarious localities.

In the age-groups also we note (column 4 : ' Cases with symptoms or history ') that the highest parasite rate occurs in the age-group 13 months–4 years, just as it does in the Macedonian population generally, as shown by

TABLE I

Summary of results of survey of malaria morbidity in five village groups

Villages	Population	Age-groups	Cases with symptoms or history		Completely incapacitated				Partially incapacitated				History only			
			Total no.	Per cent. blood positive	Total no. of cases	Per cent. of cases in whole population	No. with positive blood	Per cent. in whole population of cases with positive blood	Total no. of cases	Per cent. of cases in whole population	No. with positive blood	Per cent. in whole population of cases with positive blood	Total no. of cases	Per cent. of cases in whole population	No. with positive blood	Per cent. in whole population of cases with positive blood
A. Epidemic	140	All ages	8	75	4	2·8	4	2·8	4	2·8	2	1·4				
B. Highly endemic	2,089	12 months or under	24	62	1		1		4		3		19		11	
		13 months–4 years	45	80	4		4		10		10		31		22	
		5–10 years	25	76	6		4		4		3		15		12	
		11–15 years	11	36	4		2		1		0		6		2	
		16 years or over	68	47	24		9		17		9		27		14	
		All ages	173	61	39	1·8	20	0·9	36	1·7	25	1·1	98	4·6	61	2·9
C. Endemic	1,500	12 months or under	6	33	2		1		0		0		4		1	
		13 months–4 years	9	66	4		4		2		1		3		1	
		5–10 years	12	41	2		1		5		3		5		1	
		11–15 years	5	20	2		1		1		0		2		0	
		16 years or over	15	46	7		4		7		3		1		0	
		All ages	47	44	17	1·1	11	0·7	15	1·0	7	0·4	15	1·0	3	0·2
D. Diminished endemicity	2,978	12 months or under	10	10	0		0		1		1		9		0	
		13 months–4 years	21	38	1		0		7		3		13		5	
		5–10 years	23	17	1		0		4		2		18		2	
		11–15 years	9	33	0		0		1		0		8		3	
		16 years or over	35	20	11		2		5		1		19		4	
		All ages	98	23	13	0·4	2	0·06	18	0·6	7	0·2	67	2·2	14	0·4
E. Mountain villages	1,439	All ages	29	0	4	0·2	0	0·0	7	0·4	0	0·0	18	1·2	0	0·0
All villages	8,146	All ages	355	43	77	0·9	37	0·4	80	0·9	41	0·5	198	2·4	78	0·95

our numerous parasite surveys. In the column 'History only', the correlation of total cases and endemicity is not so great, but that of parasite-positive cases is nearly the same as in the clinical groups.

In the hope of elucidating the problem, we have further analyzed our data in Tables II and III. In Table II, we have compared the parasite index

TABLE II

Village, clinical and history groups compared with respect to the parasite index

Village groups	Totally incapacitated		Partially incapacitated		No obvious signs of acute malaria		Per cent. positive blood examination in general population (corrected for age)
	No.	Per cent. with positive blood	No.	Per cent. with positive blood	No.	Per cent. with positive blood	
A.	4	100	4	50	*		†
B.	39	51	36	69	98	62	43
C.	17	64	15	46	15	20	26
D.	13	15	18	38	67	20	9
E.	4	0	7	0	18	0	‡
Total	73	50	73	56	180	42	28

* A complete history survey was not made in Lithotopos.
† School children 97 per cent.
‡ See p. 401.

of the clinical and history groups with that of the general population, as shown by the routine autumn survey of 1934. The latter index is corrected for age; that is, it represents the index one would obtain in a village among a group of persons taken at random but with the same age composition as that of the persons showing symptoms or history.

In Table III, various groups are combined for more convenient comparison. The villages with relative high endemicity are put together for comparison with the large group, D, of low endemicity; and all clinical cases are combined for comparison with those having history only. Data are arranged by age-groups.

In Tables II and III we note that the parasite index of the clinical groups is definitely higher than that of the general population, except in the small age-group, 11–15 years. In the history group we note the same tendency, except among infants 0–12 months, in village group D, where of nine infants none was blood-positive. This finding is significant. Infants, when ill of malaria, usually show many parasites in the blood; their parasite rates are high in all other combinations of the Table. So it is probable that few, if any,

of these nine infant cases were truly malaria. This fact makes us doubtful of all history cases, although very probably some of them were genuine. In any event, we cannot rely much on cases with history only in computing the general index of morbidity. In any comparison of parasite indices, we must

TABLE III

Age-groups and the parasite index compared

Village groups	Age-group	Cases partially or completely incapacitated			Cases with history only			Autumn survey of 1934: per cent. positive (corrected for age)
		No. exposed	No. positive	Per cent. positive	No. exposed	No. positive	Per cent. positive	
A, B and C.	0–12 months	7	5	71	22	12	54	25
	13 months–4 years	21	20	95	34	23	67	58
	5–10 years	18	12	66	20	13	65	60
	11–15 years	8	3	37	8	2	25	40
	16 years or over	61	29	47	28	14	50	18
D.	0–12 months	1	1	100	9	0	0	5
	13 months–4 years	8	3	37	13	5	38	9
	5–10 years	5	2	40	18	2	11	14
	11–15 years	1	0	0	8	3	37	7
	16 years or over	16	3	18	19	4	21	6

remember that the general parasite survey of a population may include many clinical cases, especially among children of pre-school age.

In sum, we see how difficult it is to make a precise morbidity survey in an endemic region. Symptoms of malaria are variable, and the classical signs, intermittent fever and rigor, are often absent in genuine cases. Illness varies all the way from a slight indisposition to an attack of great severity. We have in our equation two unknown quantities—the definition of the disease and the degree of its prevalence. We had hoped to add a new equation by comparing various village groups with each other and with a control, but the attempt was not wholly successful. The control, it is true, had but a small incidence of clinical cases, but it had a fairly large one of history cases. People are prone to exaggerate histories in hopes of a gift of quinine for themselves or their friends. The remedy makes the disease. Possibly the most worth-while result of our survey is that we show the extent of the difficulties of diagnosis and where some of them lie.

With regard to the amount of recognizable clinical malaria in the whole survey, the totals after ' All villages ' of Table I indicate less than 2 per cent. clinically ill, including parasite-positives and negatives. Among parasite-positives the percentage is under 1. If we omit all cases based on history alone, and also those of the mountain villages in Group E, we have only 2·2 per cent.

It will be remembered that these percentages represent the findings at one visit, a cross-section of the population. Five villages in the highly endemic Group B were resurveyed 20–30 days after the first survey. A comparison of the results of the two surveys, including all five villages and only clinical cases, is here given : positive in both surveys, 4 ; positive in the first, negative in the second, 64 ; negative in the first, positive in the second, 53 ; negative in both, 1,589. In a resurvey of five villages in the ' diminished endemic ' group, no cases were found positive in both visits.

From these data we may conclude that bi-monthly or even monthly visits would have detected a much larger number of sick than did a single survey. The best method, of course, is to have a good observer in continuous residence in each of a considerable number of villages. Such continuous study of only one or two villages would give valuable results, but might not be representative of a large territory. Moreover, good observers available for long-continued study are hard to find.

SUMMARY

A malaria morbidity survey of 8,146 persons in 18 villages of Eastern Macedonia was made by house-to-house visits during the late summer and autumn of 1934. The villages surveyed are grouped as follows : one with epidemic conditions at the time of visit ; six in which malaria is highly endemic ; three in which malaria is endemic but in less degree ; eight in which malaria, formerly severe, has greatly diminished during the past three years, and in which transmission, as measured by the numbers and sporozoite index of anophelines and by the infection rate of infants, has become almost nil. Two mountain villages with almost no malaria are included as controls. The difference in the morbidity rates of the four endemic groups is marked, their percentage in total population decreasing with the degree of malarial endemicity as follows : 5·6, 3·5, 2·1 and 1·0, with an average of all groups of 2·2. These figures do not include cases from the mountain control villages nor cases of any group based on history only. The difficulties of evaluating clinical symptoms and blood parasite findings are discussed. It is evident that all percentages of morbidity can be only approximate. In our survey, morbidity rates are based on cases found at a single survey and represent only a cross-section of the population. Repeated surveys of some villages indicated that the morbidity rates for a whole malarial season would be much higher.

THE MANIFESTATIONS OF VITAMIN A DEFICIENCY IN MAN

BY

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CONTENTS

I. INTRODUCTION

The reason for a further contribution to the already formidable literature on this subject may conveniently be stated at the outset: there is as yet no unanimity as to the clinical and pathological effects which are connoted in the term 'vitamin A deficiency.' It is the exception rather than the rule to find human patients in whom a single vitamin deficiency can be postulated with anything approaching certainty, and hence the literature abounds with descriptions of 'vitamin A deficiency' cases in whom other food deficiencies co-exist. Thus in Nicholls's (1933) excellent account of cases of phrynoderma in Ceylon the patients' diet showed a marked deficiency of vitamins A, B_2 and D; similarly in Goodwin's case (1934) there was possibly an absolute lack of vitamin C. I fancy that deficiency of vitamin A has been accused of causing far too great a variety of symptoms and signs; some of these have already been denied (Richards, 1935), and a consideration of individual manifestations will help to relegate many others to their proper place. A survey of articles such as those of Stannus (1912), Nicolau (1918), Wiltshire (1919) and Scheer and Keil (1934) is sufficient to remind us that a single avitaminosis is something of a clinical curiosity, and hence that conclusions drawn from the study of indigents and famine sufferers may be fallacious.

The experience of carefully studying over 150 cases of apparently pure avitaminosis A (1933), followed by a study of 300 similar cases in the free

population of Teso, Uganda (1935), has led me to believe that many of the suspected sequelae of vitamin A deficiency are due to the lack of some other food factor.

II. ACCEPTED SIGNS AND SYMPTOMS OF VITAMIN A DEFICIENCY

Xerophthalmia

A dryness of the bulbar conjunctiva, with eventual formation of Bitôt's spots in the interpalpebral fissure ; usually these appear on the external side of the cornea, but in advanced cases they may be seen on the internal side also. The appearance is so characteristic and has been described so frequently that a further account is superfluous here ; it may be mentioned, however, that very early Bitôt's spots are easily overlooked unless a powerful lens is employed.* Also the recognition of the earlier skin manifestations often discloses a greater number of cases of xerophthalmia than would have been noticed in a routine examination of prison or school inmates.

The great majority of cases of xerophthalmia are due to vitamin A deficiency ; a few, however, follow local conditions, such as severe ectropion or lagophthalmos, which do not permit regular moistening of the bulbar conjunctiva (Parsons, 1931). In practice it would hardly be possible to confuse the condition aetiologically.

Keratomalacia

Prolonged vitamin A deficiency, especially in children but occasionally in adults (Pillat, 1929 ; Owen, 1933), leads to degeneration of the cornea, perforation and, in many cases, permanent blindness. Deficiency of vitamin A is the sole cause of this condition.

Night-Blindness (*nyctalopia* ; sometimes called *hemeralopia*. In view of this confusion of terms, the names derived from the Greek are best abandoned)

In cases of pure vitamin A deficiency there is frequently inability to see in a dim light after normal daylight. The condition is due to retinal fatigue, which shows itself by a delayed regeneration of the visual purple after light-stimulation. The affection has been known from the earliest times, has occurred in conjunction with scurvy among debilitated and famished communities, and has been treated empirically with raw liver in certain parts of the world for thousands of years.

Night-blindness is also a marked feature of retinitis pigmentosa, may rarely be present as a congenital affliction, and is frequently complained of by malingerers in prisons where the symptoms of vitamin A deficiency are common knowledge among the inmates.

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*I have found the electric ophthalmoscope, with a +20 lens in the aperture, of great service in the routine examination of the interpalpebral fissure of large series of cases.

In primitive communities, and especially in the course of field-work on nutrition, the presence or absence of this symptom cannot be accorded much importance ; the amount of credence to be attached to a denial of night-blindness by the negro children among whom I have been working is problematical. Many, however, state that vision in the afternoon is decidedly poor, another important sign of retinal fatigue (De Gouvêa, 1883).

Skin Manifestations (*phrynoderma* of Nicholls)

The skin of the whole body, with the exception sometimes of the face and scalp, becomes dry, harsh and irritating, and after a time the normal furrows of the skin become accentuated. In cases reported from China (Frazier and Hu, 1931) a diffuse pigmentation of the skin appeared simultaneously. The harshness of the skin is partly due to increased keratinization, and when this becomes excessive in and around the pilo-sebaceous orifices retention and inspissation of sebum occur, with formation of the typical papular eruption (Loewenthal, 1933). In children, this keratosis follicularis tends to be less marked and is often nothing more than a widely distributed ' goose-skin.' Changes in the nails are occasionally seen in old-standing cases: the nails lose their lustre, become transversely striated and exceptionally brittle.

A condition indistinguishable from acne vulgaris occurs in a large proportion of adults suffering from vitamin A deficiency ; it is noteworthy, however, that pustulation in my series of adult cases was extremely rare. The facial acneiform eruption never occurs in children who have not reached puberty, and in vitamin A deficient adolescents the profusion of the eruption seems to be definitely proportional to the degree of maturity attained.

III. DOUBTFUL SIGNS AND SYMPTOMS OF VITAMIN A DEFICIENCY

Neuritis

It has been stated that deficiency of vitamin A leads to neuritis, as shown by sensations of burning and tingling in the extremities, and by subsequent loss of the deep reflexes (Nicholls). Moore (1934) has also found evidence of retrobulbar neuritis in patients suffering from avitaminosis. In my own series I have not found a single case with either subjective or objective evidence of peripheral nerve involvement, although I have kept a sharp look-out for such signs in examining the cases found among the free population.

When we consider that involvement of the nervous system is a cardinal manifestation of both pellagra and beri-beri, and that acroparaesthesia is not uncommon in the former disease, it is feasible to assume that neuritis, whether of the limbs or retrobulbar, is a manifestation of some co-existing deficiency, not of vitamin A.

It is, however, only reasonable to recall that many cases of xerophthalmia show a diminished corneal and conjunctival sensibility, and that Mellanby found

degenerative changes in the peripheral nerves of animals suffering from vitamin A deprivation ; but, until the aetiology of pellagra is thoroughly understood, it is better not to assume that similar nerve degenerations occur in human cases Pellagra is mentioned here as there is some ground for believing that lack of vitamin A is a predisposing cause.

' Sore Mouth ' and Perlèche

Nicholls (1934) found a high percentage of cases with sore mouth among the vitamin A deficients in Ceylon. His description may be quoted fully : ' The standard adopted has been patches of superficial erosion of the mucous membrane of the tongue or lower lip, or its later stages, when the tongue becomes red and glazed. These patches are red and are in marked contrast to the unaffected parts of the tongue which show the whitish *duvet* of the slight normal fur.' One of his illustrations shows a typical perlèche at the angles of the mouth. It is interesting to read that Williams (1933) found a co-existence of erosion at the angles of the mouth with stomatitis in her patients, whom Stannus (1934) considers with every justification to be infant pellagrins.

Smith (1932) describes and pictures the same affection, glossitis and perlèche co-existing ; he mentions that the latter is attributed by some to a vitamin deficiency. It is now generally believed that perlèche is due to infection with *Saccharomyces albicans* or other yeast-like fungi ; it is interesting to speculate how far such a fungus infection may depend on previous food deprivation. The conditions whose incidence was especially reduced in Mackay's (1934*b*) series of vitamin A-fed babies were :—napkin rash, intertrigo, external otitis, septic spots, dribbling rash, thrush, boils and whitlows—all of them diseases which, in the young, are frequently caused by infection with the normally saprophytic *Saccharomyces albicans.*

Neither in adults nor in children suffering from vitamin A deficiency have I seen a single case of stomatitis or perlèche ; one child out of 300 vitamin A deficient children stated that he had had a sore mouth some weeks before ; there was no sign of stomatitis when I saw him suffering from xerophthalmia and marked cutaneous changes. I can produce no positive evidence, therefore, that either stomatitis or perlèche is due to deficiency of vitamin A, and prefer to regard their occurrence in Nicholls's cases as evidence of early pellagra, or as purely accidental.

' Itchy Scrotum '

This has been described by Moore as being due to a vitamin deficiency. Esquier (1928) gives a full account of its occurrence in Senegalese soldiers, in many cases associated with ' nodular prurigo or lichen obtusus.' A condition of the scrotum identical with that described by Esquier is attributed by Smith to moniliasis, and the cutaneous manifestations of vitamin A deficiency might easily be described as ' lichen obtusus.' I am not, however, suggesting any

causal relationship between the three conditions, and, in fact, have seen no cases of itchy scrotum among my proved vitamin A deficients.

Diarrhoea and Dysentery

Affections of the intestinal tract are certainly not a part of the clinical picture of mild vitamin A deficiency, as my enquiry among children of school age shows. Mackay's figures for diseases of the digestive tract also show no difference between the control group and those given an additional ration of vitamin A. Analogy from animal experiments, again, must not be given too much weight, for the incidence of diarrhoea varies with the animal used, being, for instance, exceptionally high in monkeys and low in guinea-pigs. Deprivation of vitamin A does (in animals) lead to changes in the mucous membrane of the bowel, but it is doubtful whether diarrhoea and dysentery occur exceptionally frequently in human cases of pure vitamin A deficiency.

A study of appendix IV of Mitchell's (1933) paper in conjunction with the remainder of his article is of great interest. In the Kampala prisons during 1927 and 1928 both pellagra and xerophthalmia were rife, and in those years the death-rate from dysentery alone was 12 and 23 per thousand inmates. In 1929, 1930 and 1931 there were no cases of pellagra, though xerophthalmia and other signs of vitamin A deficiency persisted ; in those three years the death-rate from dysentery was 4, 1 and 1.

I suggest, therefore, that diarrhoea and dysentery, when reported in cases of vitamin A deficiency, are often due to unrecognized, co-existing mild attacks of pellagra.

General Infections

Vitamin A is no longer universally believed to be the anti-infective factor, although its effect on epithelia may be to prevent the entrance of undue numbers of pathogens. In my own series of cases I saw no reason to believe that susceptibility to systemic infections bore any relation to vitamin A deficiency. Mitchell's figures, too, show the highest incidence of pneumonia during the pellagra years and a gradual decline to a normal figure subsequently, though vitamin A deficiency persisted.* In Mackay's compared groups, too, the difference in general morbidity was so slight as to be negligible.

Cutaneous Sepsis

It is generally agreed that vitamin A deficiency favours the development of septic dermatoses. Again, however, my observations are at variance with the general belief ; the cutaneous lesions of vitamin A deficiency are, in my experience, secondarily infected in extremely few cases, and even the facial acne seen in these patients seems to pustulate more rarely than the common non-dietetic acne vulgaris.

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*The sanitation of the prison, however, was improved at about this time.

Among those who describe the frequent occurrence of subcutaneous abscesses, impetigo contagiosa, furunculosis and ulcers are Pillat and Frazier and Hu ; in their cases the possibility of other food factors being deficient was not excluded. Similarly, those authors, from Hippocrates onwards, who have noted the association of night-blindness or xerophthalmia with cutaneous sepsis, have (to my knowledge) adduced no evidence that the diet of their patients was deficient in vitamin A and complete in other respects. The findings of Mackay, however, must be taken into account ; her conclusions are that ' the effect of . . . slight vitamin A deficiency on the health of infants is to diminish their resistance to infections of the skin such as occur with sore buttocks, intertrigo and dribbling rashes. . . .' It was by no means proved that such effects were more than mechanical, or, if infective, that they were due to bacteria and not to monilia or other fungi.

Changes in the Hair

Bleaching and falling of the hair have been recorded in vitamin A deficiency ; again I must submit that none of my cases has shown either of these signs, and that both are well-known concomitants of pellagra.

IV. DISCUSSION

Let us assume that keratomalacia, xerophthalmia, night-blindness and phrynoderma are positive signs of vitamin A deficiency. Let us remember further that a deficiency of vitamin A alone is uncommon in human subjects. Symptoms and signs, therefore, other than those mentioned above may well be due to the lack of food factors other than vitamin A. Moreover, if such signs and symptoms are uniformly absent, or are present only in normal rates in a series of 300 cases showing positive evidence of vitamin A deficiency, a common causation must be doubtful. It may be that sepsis, systemic infections, ' sore mouth ' and other phenomena are signs of a vitamin A deficiency more profound than was present in my cases ; I prefer, however, to believe that the occurrence of such phenomena points to the co-existence of pellagra or other dietary deficiencies.

V. CONCLUSIONS

1. Vitamin A deficiency in man manifests itself in certain ocular and cutaneous symptoms and signs.

2. Neuritis, diarrhoea, dysentery and cutaneous infections with pyogenic organisms, occurring in human cases of vitamin A deficiency, are probably due to lack of other food factors. If deficiency of vitamin A is a predisposing cause to pellagra, then the confusion is easily understood.

3. ' Sore mouth,' ' itchy scrotum,' perlèche, changes in the hair and increased susceptibility to general infections are not sequelae of vitamin A deficiency in man.

My thanks are due to the Honourable Dr. W. H. Kauntze, M.B.E., Director of Medical Services, Uganda, for permission to publish this paper.

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Addendum

Since the preparation of this paper, I have seen an abstract of an article by Dr. Helen Mackay (' Dietetic deficiencies and susceptibility to infection,' *Lancet*, vol. 227, 1462, December 29th, 1934), many of whose conclusions support those I have formulated.

NOTES ON AN EAST AFRICAN VESICANT BEETLE, *PAEDERUS CREBRIPUNCTATUS* Epp.

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The vesicating beetles of East Africa have not, so far, been assigned a rôle of economic importance, either as active agents in the causation of diseases, or in that the action of their body-juices acts as a predisposing cause to other diseases. However, the inflammatory condition of the skin resulting from the action of the body-juices of these insects must be of some considerable clinical importance in tropical countries, and may be followed by complications. Judging by the number of Europeans who suffer from the results of crushing these insects on their bodies, and the alarming total of conjunctivitis cases occurring in certain native tribes, it appears that some attention should be given to the possible economic rôle of this group in connection with some of our problems.

The common species in and around Nairobi is *Paederus crebripunctatus* Epp., which is well-known colloquially to the European residents as ' Nairobi eye ' or ' Nairobi fly,' signifying its connection with the prevalence of getting body-juices into the eyes. Strangely enough, the native population have no knowledge of the effects of handling these insects nor of any subsequent ill effects which arise from crushing them on varied parts of the body. It is a small insect, about 1 cm. long, but conspicuous on account of its black head, red thorax and green elytra, and from the fact that the greater portion of the abdomen is red, with the last two segments black. Whilst walking or resting, the tip of the abdomen is usually upturned ; it is a strong flier and intensely active.

The numerical fluctuations and periodicity displayed by the species is a remarkable phenomenon in certain areas of the Nairobi district. One of the writers had his first experience with the insect after occupying a bungalow situated about 300 yards from a small stream. In May, 1930, a few of these insects had been observed to be attracted nightly to the lights of the house, but suddenly in the following month, after some heavy showers of rain, an invasion of considerable magnitude took place. At first the verandah walls and ceiling were blackened by the multitude, and then came the invasion of the interior of the house. They crept in through every available crack in the doors and

windows. Being attracted to the lighted room, they did not invade other rooms where the lights were out ; but the light itself was surrounded by dense clusters of the insects. On this occasion the insects were destroyed by spraying a paraffin-pyrethrum mixture directly on to them.

One of the present writers had heard of the painful effects following rubbing or crushing these insects on the skin, and was aware that several people were suffering from acute inflammation of the eyes as a result of touching them after crushing the insects, a condition apparently well-known to the residents of the area and popularly termed ' Nairobi eye.' In consequence, two of the insects were crushed on the back of the hand and the effect was observed. Only the juices from the genital opening were emitted when slight pressure was put on the abdomen. Prior to retiring for the night, the part of the hand on which the insects had been rubbed was thoroughly washed with a carbolic soap, and even after some days had elapsed no visible signs could be observed that the juices had had any effect on the skin of the hand. However, as the writer slept under a mosquito net, it can only be surmised that during the night he had unconsciously rubbed his eye, as the following day there was slight inflammation of the eyelids, which gradually became extremely painful, suppuration setting in. This condition persisted for about two days, when the frequent applications of fomentations reduced the inflammation slightly ; the eye, however, remained very painful for a considerable number of days, and was sore for weeks afterwards.

Since that time, observations have been made whenever opportunity offered, and a few experiments have been carried out. It has been found that the insect is very common during periods following either the short or the long rains, such periods being followed by seasons of high winds and dust, commonly occurring in July, August and September or December and January. It is also noteworthy that these are the periods during which crops are reaped and stored, and when the countryside is covered with the ripened dense vegetative growth following the rains. Also, many indigenous and exotic trees have their pollinating periods just prior to the time when the maximum density of the insect population is observed. It may be relevant to repeat at this stage the well-known fact that the density of productiveness of vegetation in Africa is almost entirely dependent on rainfall. Observations made on several varieties of trees have shown these insects to be pollen-feeders, mainly on the pollen of wattle and coffee trees. But the main feeding grounds are undoubtedly the flowering heads of grasses, beneath which also have been found the main breeding grounds, particularly along the edges of streams.

Having ascertained the main feeding and breeding grounds to be mostly composed of the flowering heads of grasses growing along the edges of streams, the explanation for the periodic fluctuations can thus largely be accounted for by the abundance or otherwise of these grasses. During seasons of heavy rainfall, such as 1930 proved to be, the wild grasses in all parts of the country

were most luxuriant; but in a season such as 1933, when there has been a drought and grasses are naturally scarce, it is most difficult to obtain a specimen of this insect. Heavy rainfall seasons, producing a greater amount of food in the flowering heads of grasses, can thus be regarded as those which are most productive of numbers of insects and give a greater survival rate.

EFFECT ON EUROPEANS

The irritant powers of *P. crebripunctatus* have been tested on Europeans and Africans, and the results have been in most instances similar. Most of the European cases reported or known to have suffered from conjunctivitis give the same history. The following case, though rather exceptional, is given as an instance of the potency of the juice of these insects and shows the care which must be taken after touching them. A European lady killed one of these insects on a mirror by crushing it with a piece of cotton wool, the wool being then discarded and a fresh piece obtained to carry out some further facial treatment on which she was engaged. Next day her eye became sore and inflammation set in; and by the second day a severe conjunctivitis was experienced. It appears that some of the juice from the crushed insect had somehow touched her eye, though the lady vows that she did not touch her eye with her exposed finger on that occasion.

Children are also very liable to suffer from blistering on various parts of the body, and in one case, where a child had crushed the insect on her shoulder and drawn her hand across her chest, blistering occurred along the whole path of her hand. It has also been noted that children, owing to their habit of playing on grass in gardens, are liable to conjunctivitis or blistering on various parts of the body, and the real cause is often overlooked.

Most of the experimental work carried out with these insects has followed on the lines of Strickland's (1924) investigations, and the results may be briefly summarized as follows. When the insects are allowed to wander freely on the surface of the skin, there is no result, nor when the insect is worried by probing. When the insect is crushed and rubbed into the skin of the hand, there is usually no reaction; on one or two occasions a slight lesion has been observed on the more tender parts, such as the wrist joints. On the forearm of Europeans, typical reactions were obtained after rubbing in the crushed insects, and, after an incubation period of about 24 to 48 hours, scattered bullae made their appearance, which finally coalesced and formed a large single blister.

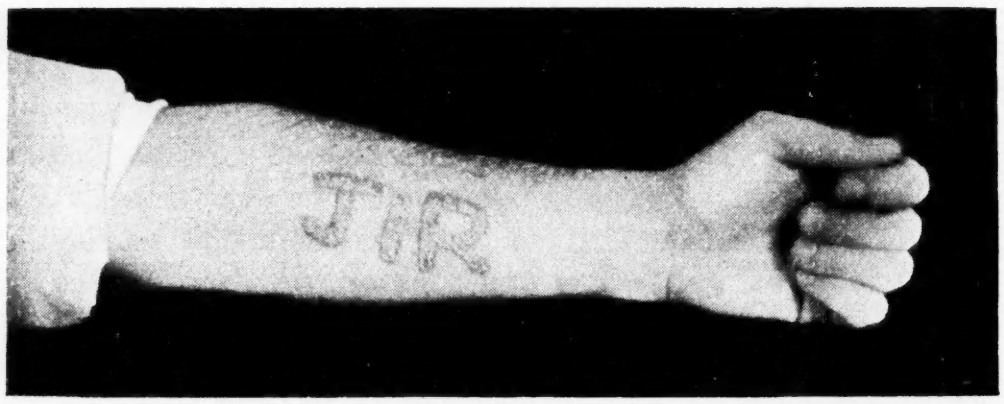
In view of these facts and of the statements usually encountered that a cut on the skin is essential for the entry of the juices, and that the action in crushing and rubbing into the skin of the chitinous skeleton produced small cuts which allow such penetration, the following experiment was carried out.

Seven *Paederus* adults were ground up in 10 c.cm. of 2 per cent. alcohol, diluted with distilled water to avoid the risk of contamination, and the whole

was filtered. The initials J. I. R. were delineated with a perfectly smooth-ended glass rod on the anterior surface of the left forearm.

3.8.32 : Initials delineated on forearm with filtrate.
4.8.32 : Initials reddened and easily distinguishable.
5.8.32 : Inflammation sets in.
6.8.32 : Blistering stage with constitutional disturbance and slight headache.
7.8.32 : Pains experienced in most parts of the body, particularly in the joints and back. Severe headache. Blisters large and fierce and almost completely coalesced.
8.8.32 : Photograph taken. Blister stage acute, complete coalescence and swollen. Treatment with $MgSO_4$ compress commenced.
9.8.32 : Few blisters left. Slept well and pain greatly subsided.
10.8.32 : Blisters disappear. No constitutional disturbances remaining.

The initials, covering an area of several square inches, gave a cicatrix which persisted for eight months.



Photograph by M. H. Fox, Esq., Government Analyst, Kenya
Photograph showing blisters caused by an application of the filtrate to the forearm.

After this painful experiment, only very small areas of the skin were experimented with thereafter. Crushing the insects with a glass rod and smearing small areas of the skin with the juices gave a much reduced incubation period, and bullae appeared in 24 hours, with a complete coalescence on the second or third day. These areas, if allowed to burst, left intensely sore and raw areas.

EFFECT ON AFRICANS

A native volunteer was experimented with at the same time as the European, using some of the same filtrate as in the previous experiment. In his case, no blisters were caused on the forearm as in the case of the European, but a lesion similar to that obtained on the hand was made, followed by a cicatrix which remained for some time. The failure to produce bullae is considered to be due to the hardened exposed skin, as this African had never worn sleeves.

However, when the juice was placed on other parts of the body which are not usually exposed, blistering was easily produced.

Several hundred insects were then collected from grasses near a river and dried in an oven, the dried mass being afterwards pulverized and some of the

powder rubbed on to the skin of guinea-pigs and human beings. The results demonstrated that even desiccated insects are capable of causing blistering—a fact which may well be of great importance in view of the suggestion that these insects have a rôle in the aetiology of some of our diseases.

TREATMENT

In *Paederus crebripunctatus*, like all the *Meloidae*, the active principle of the excreted juices is cantharidine, cantharides normally containing 0·5 c.cm. to 0·7 c.cm. of the principle, which in minute quantities is capable of causing a blister. From the earliest times plasters have been prepared by extracting beetle-juices, the better-known vesicating beetles occurring in the family *Lyttonae*. ' Spanish fly ' and plasters made therefrom are used as counter-irritants.

In the lesions caused by *Paederus*, a sequence of events is noticed, closely coinciding with that seen in blistering by cantharides plaster, but much more intense. Application of the liquid extract from the beetle shows at first an area of erythema, then formation of minute bullae, followed by the coalescence of the bullae to form one large blister covering the entire area touched by the poison.

Although in and around Nairobi the insect is known as ' Nairobi eye,' because the most characteristic lesion is seen in or about the eye, it is not thought that the insect has any predilection for the eye. A person, feeling an insect on the face in the region of the eye, brushes it off with a brisk movement, usually smearing the remains right across the face, and then, to ensure its complete removal, gives an extra rub or two. A thorough application of the crushed insect is thus given, as the instinctive movement in rubbing the face is to start at the eyes and to rub away from them. It will thus be expected that the situation of the lesions will be most frequent on those parts of the body that the eye cannot see, which is always found to be the case, the face and the back of the neck being the commonest sites.

Lesions caused by the insects can be and often are temporarily most disabling, as the conjunctivitis caused is intense and the sympathetic lachrymation of the unaffected eye causes temporary loss of vision from both eyes. No cases of actual blindness from the insects have been reported, as is believed to be the case in *P. amazonicus* in young children.

The symptoms noted in his own case by one of the present authors after blistering an area on his arm, were pains in the neck and back and a generalized headache. These symptoms disappeared immediately on the application of a cold saturated magnesium sulphate compress. No evidence is forthcoming of polyuria being caused or of the presence of albumen or casts in the urine.

Until recently it has been customary to treat the skin lesions by the application of soothing unguents. The authors have found that the exhibition of any greasy preparation has the immediate effect of aggravating the raw areas and in some cases of causing a spread of the bullous area. Such preparations as

unguentum zinci, Lassar's paste, acriflavine and cold cream are useless in the treatment. The authors have tested a cold compress of saturated solution magnesium sulphate on the unburst blebs and on the raw areas formed subsequent to the bursting of the bullae, which had the effect of immediately relieving all pain and shortened the time taken in healing the ulcers. Following the $MgSO_4$ compresses, calamine lotion or any dusting powder can be applied—in fact, it would appear that after the exhibition of $MgSO_4$ any treatment can be adopted so long as greasy preparations are avoided.

The lesions show a marked tendency to cicatrix formation, and, as previously mentioned, one of the authors had well-marked scars on his forearm for eight months after the initial blistering.

In considering the magnesium sulphate treatment, there appears to be a definite specific action exercised on the poison apart from the hypertonicity of the solution, which would appear to be analogous to the action of intravenous $MgSO_4$ solution on the toxin of the red-back spider of the Philippine Islands.

SUMMARY

1. *Paederus crebripunctatus*, a very potent vesicating beetle, increases to enormous numbers in Kenya after seasons of heavy rainfall which are productive of a luxuriant growth of grasses, upon the flowering heads of which the insects feed.

2. The insect is well known to the European population as 'Nairobi eye,' owing to the conjunctivitis produced when the juices of crushed insects are rubbed into the eye.

3. The toxic juices are excreted by the genital glands and the fluid easily vesicates unbroken skin.

4. The vesicating principle is not destroyed by heat or desiccation.

5. $MgSO_4$ compresses give immediate relief for the condition.

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STUDIES ON CHEMOTHERAPY IN BIRD MALARIA*

IV.—FAILURE TO PROMOTE DRUG-RESISTANCE IN *PLASMODIUM CATHEMERIUM* BY PROLONGED ADMINISTRATION OF QUININE OR PLASMOCHIN

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I. INTRODUCTION

It has often been assumed that strains of malarial parasite are capable of developing a drug-resistance during and as a result of treatment. However, justification for this belief appears to rest far more on a basis of analogy with the well-known and well-established powers of attaining drug-resistance shown by certain other protozoa, notably the pathogenic trypanosomes, than upon any unequivocal, direct proof. The numerous observations in this connection which have been advanced by investigators of human malaria in many parts of the world will not be listed or discussed here, and one may, perhaps, be permitted to pass by the greater part of this work and argument in the general conclusion that the matter has still remained somewhat in doubt. Among studies of the subject in bird malaria, however, special attention might be drawn here to the claims made by Et. and Ed. Sergent (1921*a*, 1921*b*) and by Kritschewski and Rubinstein (1932) of having elicited and demonstrated acquired drug-resistance in *Plasmodium relictum*.

The Sergent brothers state that, in an infected bird which was being subjected to a prolonged course of quininization, parasites began to increase in the blood at the end of 9 months, in spite of the continuation of treatment; this accumulation of parasites the authors ascribe to the development of quinine-resistance, which is said to have been maintained for two succeeding passages, but was, for the greater part, lost on the third. A further interesting conclusion reached by these workers as a result of the same researches is that, following on the quininization of infected birds over periods of 1 to 6 months, the virulence of the parasites is liable to be temporarily attenuated, so that for two or three succeeding untreated passages only milder infections arise. These findings of the Sergent brothers will be referred to in more detail in a later section of the present contribution.

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*These investigations were carried out during the tenure of a Rockefeller Medical Fellowship of the Medical Research Council of Great Britain. The author is much indebted to Dr. W. H. Taliaferro for his valuable advice, and to Dr. Clay G. Huff for many helpful suggestions. The work was aided by financial support from the International Health Division of the Rockefeller Foundation and the Logan Fund of the University of Chicago.

Kritschewski and Rubinstein claim to have demonstrated the development of quinine-resistance by the following means :—Infected birds were subjected to a short course of quinine treatment, commencing on the day after inoculation, and the consequent protraction of the incubation period was noted ; this was found to be less in infections by parasites which had, in previous passages, been exposed to quinine treatment, than it was in other cases, and the writers attribute the difference to the attainment of quinine-resistance by the parasites of the previously treated infections. Following up this work, Kritschewski and Halperin (1933) found that the form of quinine-resistance demonstrated by the above technique is sometimes, though not invariably, retained on passage through *Culex pipiens*. The work is of much interest, but it suggests to one that, if true quinine-resistance may really be produced in the parasites of bird malaria as easily and as rapidly as is claimed by Kritschewski and his collaborators, then, in a strain which is ordinarily sensitive to quinine treatment, it should surely be capable of being demonstrated in a more direct way. The strain of *P. cathemerium* used in the experiments reported below was subjected to very much more rigorous and protracted quininization than was applied by Kritschewski and his co-workers to their strain of *P. relictum*, and yet, as will be seen, no clear and direct evidence of the acquirement of quinine-resistance could be adduced ; acceptance of the conclusions of Kritschewski and his colleagues would, therefore, involve now the question whether quinine-resistance may not develop considerably more readily in one species or strain of malarial parasite than in another.

II. EXPERIMENTAL WORK AND DISCUSSION OF RESULTS

If protracted treatment of bird-malaria infections by a particular drug is indeed capable of provoking resistance in the parasites, then one must reasonably expect the new character to become apparent upon treating the infections of other birds subsequently inoculated with those parasites ; the later infections should prove to be more refractory to treatment than is ordinarily the case. This was tested in the following experiment :—

Three canaries, A, B and C, were inoculated with a strain of *P. cathemerium*, and the resultant infections were then exposed to daily treatment by 1 mgm. quinine hydrochloride intraperitoneally per 16·5 gm. bird, for 63, 100 and 137 days respectively. In fact, the parasites of two of these infections had already, in previous passages, been subjected to some considerable quinine treatment, for the parasites of bird A had been exposed in two previous passages, during 28 days, to an aggregate dosage of 26 mgms. quinine, and those of bird B in four previous passages, during 60 days, to an aggregate of 39 mgms. quinine. During the entire period of infection and treatment of birds A, B and C, blood films, examined daily or every second day, showed only occasional parasites, while there was at no time any significant increase in their number. In order to detect any possible quinine-resistance developed in the parasites as a result

TABLE I

Showing failure to produce quinine-resistance in *P. cathemerium* by daily intraperitoneal treatment of infections with 1 mgm. quinine hydrochloride for respective periods of 63, 100 and 137 days

Original treated infection	Duration of original infection and daily treatment	Blood then inoculated intravenously into	Treated (T) or untreated (U)	Number of parasites per 10,000 red cells — Day of infection and of treatment															
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Bird A*	63 days	Bird 3	T			–	–		–	–	–	–	1	–		–	2	D	
		Bird 6	U			1	4		62	112	8	1		4			3		
Bird B*	100 days	Bird 208	T			–	–	–		–	1	–	–		–		–	–	–
		Bird 209	U			–	–	–		2	93	440	1,580		4,100		3,500	5,600	D
Bird C	137 days	Bird 230	T				–	–	–	–	–	–	–	–	–	–		–	
		Bird 231	U				2		87	520	1,860	1,130	1,540	2,460	3,760	D			

* The parasites of Bird A had already been subjected to an additional 26 mgms. quinine treatment during 2 previous passages, and those of Bird B to 39 mgms. during 4 previous passages.

D = died.

of the protracted treatments of birds A, B and C, the latter were therefore killed, and two other canaries infected from each case, equal amounts of blood being used for each bird in a pair, one of which was then similarly treated daily by 1 mgm. quinine hydrochloride intraperitoneally while the other remained untreated as a control. The course of these six infections was followed by examining blood films daily and estimating the approximate number of parasites per 10,000 red cells, with the results shown in Table I. It will be seen (birds 3, 208 and 230) that the parasites were still strongly sensitive to quinine, in spite of their previous exposure to daily therapeutic treatment by the drug for respective periods of at least 63, 100 and 137 days.

These results, although suggestive, certainly cannot, however, afford a conclusive basis for the standpoint that the parasites of bird malaria are incapable of acquiring a resistance to quinine. Apart from the fact that a considerably longer period of treatment than 137 days may be required in order for quinine-resistance to become established, it must be borne in mind that, during a prolonged course of quininization, the factor may possibly come into play of an habituation to the drug arising on the part of the host, involving a greater destruction of quinine in its tissues (Teichmann, 1917), or a diminished power of absorbing the drug by the red cells (Schilling and Boecker, 1919), than is the case with a non-habituated host. If some such factor does operate, then it would, of course, imply that the parasites latently infecting a host during a prolonged and unbroken period of quininization become less and less exposed to the drug as the treatment continues, and so may hardly be given sufficient opportunity to acquire an appreciable resistance. It was decided, therefore, to subject the strain of *P. cathemerium* to prolonged periods of daily quinine treatment in a succession of subinoculated hosts, each of which supports the infection for a short period before the next passage, instead of administering a protracted course of quinine to a single case ; in this way it would be possible to eliminate unknown factors introduced by the reactions of the host itself to a long-continued treatment. These particular experiments were conducted in the following manner :—

Two canaries were infected, one being treated with quinine daily, starting on the 1st day of the infection, and continuing for 2 or 3 weeks, after which it was killed, and as much blood as could be obtained from it inoculated into three other clean birds. Of these latter, two were then treated by quinine in like manner to their predecessor, while the other remained untreated for comparison. At the end of a further 2 or 3 weeks, one of these treated birds of the 2nd passage was killed, and the same cycle of inoculation, 2 weeks' treatment (one infection remaining untreated) and sacrifice of a treated bird, was carried out for three birds of the 3rd passage. The cycle was repeated thus for a large number of successive passages, and could, of course, be carried on indefinitely. For a number of the passages, though, only two birds were subinfected, and in these cases one was treated by quinine, the other remaining untreated ; and in some

TABLE II

Showing results of attempt to promote quinine-resistance in a strain of *P. cathemerium* by daily administration of 1 mgm. quinine hydrochloride during 34 passages, over a period of 511 days

Passage No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
No. of days strain has been exposed to quinine before inoculating for next passage	21	36	50	65	80	95	109	123	138	151	166	181	200	221	235	246	259	271
Highest parasite-count* in treated birds ...	2	86 1	318†	68	39	3	51	2	0	12	0	1 1	8	10 1	162†	1,470< 2,110<	§22 §0	4 D
Highest parasite-count* in untreated birds ...	2,300	1,020					1,150			2,090<	610	1,540	132	3,540<	840	494	§3,380	835

TABLE II (*continued*)

Passage No.	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	33	34	34
No. of days strain has been exposed to quinine before inoculating for next passage	287	303	317	336	351	366	380	392	407	423	437	451	465	479	493	502	511	511
Highest parasite-count* in treated birds ...	1	15 D	19 D	175 10	4 2	0 3	1 D	480<† 	§1 §D	17 14	84	5 2	218† 106	138 2	15 2	§0 §0	2 4	§0 §0
Highest parasite-count* in untreated birds ...	1,790		1,090	2,450	460	880	590	2,160	§1,270	816	1,540	1,140	3,320<	588	218	§5,120<	396	§268

* Approximate number per 10,000 red cells.

† Bird has been very weak and unhealthy since inoculation and start of treatment ; cause uncertain.

< Bird died or was killed at a time when the number of parasites was rising ; in all other cases the parasite-count number given represents a peak.

§ Bird infected by intramuscular inoculation ; all others by intravenous route.

D = Bird died within 10 days of inoculation.

TABLE III

Showing results of attempt to promote quinine-resistance in a strain of *P. cathemerium* by daily administration of 1 mgm. quinine hydrochloride during 15 passages, over a period of 272 days

Passage No.	1	2	3	4	5	6	7	8	9	10	9	10	11	12	11	12	13	14	15
No. of days strain has been exposed to quinine before inoculation for next passage	15	29	43	57	71	85	99	113 141	127	140	155	169 204	190	202	218	232	244	258	272
Highest parasite-count* in treated birds	§0	14 1	36<† 2	3 63<†>	11	14 4	49	11 7	113†	15	2 D	40 7	10	1	1 D	4 2	11	35<†>	2 D
Highest parasite-count* in untreated birds	§640	210	810	4,500<	255	302	4,000	495	540	740	162	1,690	1,780	170	745	122	47<		850

* Approximate number per 10,000 red cells.

† Bird has been very weak and unhealthy since inoculation and start of treatment ; cause uncertain.

< Bird died or was killed at a time when the number of parasites was rising; in all other cases the parasite-count number given represents a peak.

§ Bird infected by intramuscular inoculation ; all others by intravenous route.

D = Bird died within 10 days of inoculation.

few cases the passage comprised only a single quinine-treated infection, there being no accompanying untreated infection. All subinoculations were, however, invariably made from *treated* infections. It is clear that, if a resistance to quinine can arise in a strain which is thus persistently plied with the drug, then the time should come when the treated bird of a passage must show a definite acute phase of infection, in spite of its treatment, and this should recur repeatedly in the subsequently subinoculated treated birds, and should tend to approximate in severity to the acute phase in the untreated subinfections.

The treatments throughout were by 1 mgm. quinine hydrochloride per 16·5 gm. bird intraperitoneally, and the course of the various infections was again gauged by estimation every day, or every second day, of the approximate number of parasites per 10,000 red cells. The subinoculations were always made by using infective material in approximately equal amounts for each bird in each particular passage.

The results of two such prolonged experiments are given in Tables II and III. The former Table summarizes observations made almost daily upon the strain over a period of 511 days (i.e., 1 year and 5 months), during which period it was passaged 34 times, and underwent an unbroken course of daily treatment by 1 mgm. quinine hydrochloride. Table III summarizes a parallel series of observations during an unbroken period of daily quinine treatment of 272 days (i.e., 9 months), covering 15 passages.

In reference to these tabulations, it must be explained that in the column marked 'Highest parasite-count in treated birds,' where two numbers appear for any particular passage, this means that two infections were treated by quinine in that passage, the two figures representing the highest number-counts reached in each case ; the inoculations for the succeeding passage were made (with the few exceptions to be mentioned below) from the bird whose infection is represented by the upper figure of the two. To take an an example at random from Table II, passage 23 comprised 3 infections, which were simultaneously derived from that treated infection of passage 22 which had reached a peak of 175 parasites per 10,000 red cells. Of the 3 infections of passage 23, 2 were treated, and their parasite-counts did not reach above 4 and 2 parasites respectively per 10,000 red cells ; the other infection remained untreated, running to a peak number-count of 460. The treated infection which showed a maximum parasite-count of 4 per 10,000 red cells was sacrificed, its blood being divided evenly for infection of the 3 birds of passage 24.

The few exceptions, mentioned above, to this system occurred as follows :—

In Table II, passage 33 comprised 6 infections, 3 made by intravenous and 3 by intramuscular injection of blood, equal amounts being used in each case, from the treated bird of passage 32 whose infection had run to 138 parasites per 10,000 red cells. Similarly, passage 34 included 6 infections, which arose from 3 intravenous and 3 intramuscular inoculations (equal inocula for each case) from the treated infection of passage 33 which had shown 15 parasites per 10,000 red cells. In Table III, both the treated infections of passage 8 were used for subinoculations, the one after 14 days, and the other after 42 days of continuous treatment and observation ; again, both the treated infections of the later passage 10 were used for subinfection, the one after 14 days and the other after 49 days of treatment and observation.

Since the duration of many of the infections was only 2 or 3 weeks, the question may be asked whether a longer period of daily treatment and observation might not have revealed, in the quininized birds, heavier infections than are recorded in the Tables. This was ruled out in the results of daily examination of a substantial number of infections

in which treatment was continued for periods in excess of 3 weeks—in fact, for various periods up to 7 weeks.

By directing one's attention, therefore, along the two lowest columns of Tables II and III, one may see at a glance the extent to which the strain, quininized daily over two separate periods of 1 year and 5 months, and 9 months, was able to manifest itself, from passage to passage, in the face of the continuous and unbroken course of treatment, and one may compare this with the severity of infections produced at the same times by the same quininized strain in the absence of treatment.

It may be clearly deduced from the results of these experiments that the strain did not acquire any degree of constant drug-fastness during its prolonged periods of exposure to the effects of quinine treatment. It is true that in a number of cases (see, for example, Table II, passages 3, 26 and 31) moderately severe infections were attained by treated birds, but these infections were not nearly as severe in degree as their untreated companion infections; furthermore, nearly all these particular cases were quite evidently being adversely affected by some serious intercurrent cause of disability or ill-health, sometimes resulting in death, which could be accounted for neither by the malaria nor by the direct effects of quinine. That these particular moderate infections were not due to an acquirement of drug-resistance on the part of the strain should be clear from the fact that succeeding infections proved to be quite as sensitive to quinine treatment as were those at the beginning of the experiment. There was one remarkable aberration from the main trend of the results of the experiment: this occurred in passage 16 of Table II. It will be seen that the two treated infections of this passage pursued extremely severe courses, resulting in death after showing 1,470 and 2,110 parasites respectively per 10,000 red cells, in spite of the quinine treatments. These infections were no less severe than those which had been untreated, and appeared to mark the development, after 235 days' treatment, of quinine-resistance on the part of the parasites. However, the phenomenon was not once repeated during the remaining 18 passages, covering a further period of 265 days, and one must conclude that the severity of the treated infections of passage 16 is to be accounted for by some factor or factors other than an acquired quinine-resistance simply transmissible by heredity. What this factor or factors may have been, the writer is at a loss to suggest; the treatments were carried out exactly as were all others of the experiment, and one may rule out any deterioration in the quinine solution employed, since this same solution was used at the same time, with the usual therapeutic effect, in other infections. The birds did not seem to be diseased or unhealthy before inoculation, and they did not appear to differ in any way, during their infections and treatments, from any other bird carrying an uncomplicated and untreated infection. While this curious deflection from the main findings must remain obscure as to its explanation, it serves as a warning, in view of the subsequent history of the strain, against accepting a case of quinine-

resistant malaria as evidence of the attainment of true hereditarily-transmissible drug-resistance by the parasites of the infection.

As is indicated in the Tables, nearly all the transmissions were by intravenous rather than by intramuscular inoculation, and it is perhaps on this account that so many of the treated infections were by no means completely restrained as the result of treatment. It will be seen, though (Table II), that in the 33rd and 34th passages of the strain, after periods of 479 and 493 days respectively of continuous daily treatment, infections produced by the intramuscular route were completely held in check by the treatments employed.

Attention has already been drawn, in the early part of this paper, to the claims made by the brothers Sergent (1921*a*, 1921*b*) of having elicited quinine-resistance in *P. relictum* by subjecting an infected bird to a protracted course of treatment by quinine hydrochloride. The dose employed was 0·7 mgm. subcutaneously, and this was administered daily for the first 20 days of treatment, and then every alternate day for a period of nearly 9 months. (An aggregate amount of 109 mgms. quinine, during a period of 9½ months, therefore, was applied to their strain, as compared with the aggregate of 511 mgms. over a period of 1 year and 5 months in the experiment of Table II in this paper.) During the last 4 or 5 weeks of the treatment of their experimental bird, the Sergents noted an increase in the number of parasites in the peripheral blood. However, this cannot be accepted as evidence of acquired quinine-resistance on the part of the parasites, since the complicating factors suggested by the work of Teichmann (1917) and Schilling and Boecker (1919), to which reference has already been made on page 424, may well apply here; the case would then simply be one of a relapse in a bird which, while still being treated, is no longer capable of commandeering the drug against the parasites, the latter remaining fundamentally unchanged in their sensitiveness to quinine. In their preliminary note (1921*a*) the Sergents stated that the strain retained all its 'resistance' during 2 subsequent passages, but the histories of the important treated infections of these 2 passages are not given—in fact, the particular treated infections in point are not even mentioned in the longer and detailed later report (1921*b*) of the experiment; in a treated infection of the 3rd passage, an account of which is given, the quinine treatment employed did indeed hold the infection in check, although a little less easily, it is stated, than in other cases observed; the evidence of true acquired quinine-resistance provided by the published details of the Sergents' experiment is, therefore, reduced to the effect of this single treatment. In view, however, of the definite variability in response to identical treatment that may anyhow be encountered between individual birds bearing the same strain of plasmodium, this single infection of the 3rd passage, sensitive to quinine treatment as, in fact, it was, cannot be allowed alone to bear the whole onus of proof that quinine-resistance had really been acquired by the parasites of the strain.

Mention has also been made, in the introduction to this contribution, of the further interesting conclusion adduced by the Sergents, that the virulence of a strain may be attenuated after a period of quininization extending from 1 to 6 months. Such a change, or, indeed, any alteration in the character of a strain induced by drug treatment, would be of the highest theoretical, and, by implication, of great practical, interest, so that it is important for a conclusion of this kind to be supported by adequate and clear evidence. There is, however, in the first place, a certain discrepancy in the Sergents' report of the experiments on which the conclusion is based. In the earlier paper (1921*a*) it is stated that 'attenuated' infections arose from successful subinoculations from all of 6 birds quininized over periods of 1 to 6 months; but, from the later detailed account (1921*b*), it would appear that this result followed only 3 of the cases. In a control series of 9 untreated infections lasting from 1 to 6 months, subinoculations from 2 resulted in 'attenuated' infections. Furthermore, from the descriptions given by the Sergents of 5 of their 'attenuated' infections, at least 2 do not appear to have fallen outside the very wide range of variability given by the writers themselves as representing normal limits of severity for ordinary infections with their strain (height of infection—1 to 80 parasites per oil-immersion

field, sustained for 1 to 27 days). There is, then, at least some room for doubt as to whether a sound statistical basis exists for the Sergents' conclusion that attenuation of virulence of the parasites may result from prolonged quinine treatment.

Turning now to the experiments of the present contribution, for evidence of any possible alteration in virulence of the parasites of *P. cathemerium* after protracted quininization, the lowermost columns of Tables II and III, while showing that the untreated infections varied in severity within fairly wide limits, do not reveal that any recognizable attenuation of virulence on the part of the strain was produced by the treatment. A similar failure of prolonged quininization to influence the virulence of a strain of bird malaria was also brought out in a series of observations made in direct continuation of those recorded on Table III. The strain of *P. cathemerium* was passed on by intramuscular injection, after the 14 days of passage 15, into three other birds, but had become accidentally contaminated, by mosquito-bite, with a German strain of *P. relictum*, which has already been shown to be strikingly resistant to intraperitoneal quinine treatment (Lourie, 1934). Although mixed infections were thus established, the experiment was continued in exactly the same way as before for a further 13 passages, over an additional period of 255 days, during which the results shown on Table IV were obtained. The Table confirms, in the first place,

TABLE IV

Showing results of daily administration of 1 mgm. quinine hydrochloride to mixed infections by a strain of *P. cathemerium* and a German strain of *P. relictum* during 13 passages, over a period of 255 days

Passage No.	1	2	3	4	5	6	7	8	9	10	11	12	13
No. of days strains have together been exposed to quinine before inoculation for next passage ...	29	42	56	70	89	111	127	148	172	194	212	234	255
Highest parasite-count* in treated birds	382	484	284	§138	732	156	106<	224	1,530	314	860	2,180	636
	252	37<	198	§D	234	38	80	58	736<	96			D
Highest parasite-count* in untreated birds ...	660	1,920<		§216	142	33	252	5,300	89	36	112	1,360	4,320

* Approximate number per 10,000 red cells.

< Bird died or was killed at a time when the number of parasites was rising; in all other cases the parasite-count number given represents a peak.

§ Infections of first three passages were produced by intravenous inoculation ; *all* the remainder by intramuscular route.

D = Bird died within 10 days of inoculation.

TABLE V

Showing results of attempt to promote plasmochin-resistance in a strain of *P. cathemerium* by daily administration of 0·01 mgm. plasmochin during 19 passages, over a period of 293 days

Passage No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
No. of days strain has been exposed to plasmochin before inoculating for next passage ...	14	28	42	58	70	87	99	113	127	141	161	175	190	206	221	242	264	279	293
Highest parasite-count* in treated birds—																			
0·01 mgm. daily	2	0	0	2	1	1	0	0	1	0	3	1	4	0	0	0	3	0	0
0·005 mgm. daily	31	1	2	16	64	38	11	2		410		49	17	97			2	45	0
Highest parasite-count* in untreated birds ...	740	254	730	4,100	890	730<	324	418	880	1,030	282	84	220	540	480	1,570	182	1,930	237

* Approximate number per 10,000 red cells.

< Bird died when the number of parasites was rising ; in all other cases the parasite-count number given represents a peak.

Note.—All infections were produced by intravenous inoculation, and all, subsequent to those of the first passage, were made from the infection of the previous passage which had been treated by 0·01 mgm. plasmochin.

the high degree of natural resistance to quinine treatment on the part of the German strain of *P. relictum*, contrasting thus with the susceptibility to quinine of certain other strains of the same species of parasite ; secondly, the results again do not provide any evidence of the daily quininization having produced an attenuation in the virulence of the parasites (see figures for untreated infections). Such fluctuations as occurred in severity of the untreated infections fall within the somewhat wide range of variability in this respect of ordinary infections by the strain ; it may be seen that the last two untreated infections, after the *P. relictum* parasites had been subjected to daily treatment for about $8\frac{1}{2}$ months, far from giving evidence of attenuation, were among the heaviest recorded.

An attempt has also been made to promote resistance on the part of *P. cathemerium* to treatment by plasmochin.* The procedure adopted was essentially the same as in the experiments of Tables II, III and IV. The strain was passaged at intervals of 2 or 3 weeks through successive series of canaries, each passage being effected by intravenous inoculation of parasites from a *treated* infection of the preceding passage. Using a daily dosage of 0·05 mgm. plasmochin intraperitoneally, the strain was lost at the 3rd passage ; the experiment was therefore started afresh, with daily dosages of 0·01 and 0·005 mgm. respectively for the treated infections of each passage. Some passages included only one treated infection, in which case only the larger daily dose was employed. The inoculations, at each interval of about 2 weeks, were always made from the particular infection which had been treated by 0·01 mgm. of the drug, so that by the end of the experiment the strain had been exposed to uninterrupted daily treatment by 0·01 mgm. over a period of 293 days ($9\frac{1}{2}$ months), during which time 19 passages had been effected. The results of this experiment, which are summarized on Table V, did not afford the slightest evidence of the acquirement of plasmochin-resistance on the part of the parasites. There was, also, no evidence of any other alteration having been induced in the parasites of the strain as a result of the prolonged treatment.

III. SUMMARY

1. Attempts have been made to promote drug-resistance in a strain of *P. cathemerium* in canaries by the following procedures :—

(*a*) Three infections were treated by daily intraperitoneal injections of 1 mgm. quinine hydrochloride for 63, 100 and 137 days respectively. The birds were then killed, and their parasites inoculated into other subjects, in which the response to similar quinine treatment was observed.

(*b*) In two experiments the strain was passaged through a long series of birds, each passage lasting about 2 or 3 weeks, and including at least one

*The plasmochin used in these experiments was kindly supplied by the Winthrop Chemical Company, Inc., New York City.

infection treated daily by 1 mgm. of quinine hydrochloride intraperitoneally. The inoculations for each successive passage were invariably made only from a *treated* bird of the previous passage, so that by the end of the two experiments the strain, having been transmitted respectively through 34 and 15 passages, had been subjected to an uninterrupted daily treatment *in vivo* by 1 mgm. quinine hydrochloride for two separate periods of 511 days (1 year and 5 months) and 272 days (9 months).

(*c*) In similar manner to that outlined immediately above, the strain was transmitted through 19 bird-passages, during which it was subjected to an uninterrupted daily *in vivo* treatment by 0·01 mgm. of plasmochin over a period of 293 days ($9\frac{1}{2}$ months).

There is no evidence, in the results of these experiments, of an acquired and hereditarily-transmissible drug-resistance having been promoted by the prolonged treatments employed.

2. There was also no evidence that the prolonged subjection of the strain of *P. cathemerium* to quinine and plasmochin treatment had effected any attenuation in the virulence of the parasites. A similar conclusion applies in regard to an exposure of a normally quinine-resistant strain of *P. relictum* (in mixed infections with *P. cathemerium*) to daily treatment by 1 mgm. quinine hydrochloride during 13 passages over a period of 255 days ($8\frac{1}{2}$ months).

3. A critical examination is made of the claims of previous workers that drug-resistance and an attenuation in virulence of the parasites of bird malaria may be produced by prolonged quinine treatment.

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OYSTERS IN SIERRA LEONE AS POTENTIAL CARRIERS OF ENTERIC INFECTION

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From January, 1935, until the end of May, 1935, this Laboratory was asked to examine 44 patients suspected to be suffering from enteric infection or bacillary dysentery. Of these, 13 were found to be positive, by means of cultures and/or agglutination reactions ; 6 of the patients were found to be suffering from *Bact. typhosum*, 2 from *Bact. paratyphosum B*, 4 from *Bact. flexneri Y*, and 1 from a *Bact. shigae* infection. The 8 enteric patients were questioned regarding the possible source of their infection, and it was noted that 5 of them had eaten oysters within the period of incubation of the disease ; in two instances oysters formed a regular item in the patients' diet, while the others stated that they only ate them infrequently. Of the remaining three cases, two denied having eaten oysters within a period which could be associated with the onset of the illness, and in the third case, which was only diagnosed at autopsy, no history was available.

In view of these results, it was decided to make a bacteriological examination of some of the oysters supplying the Freetown market.

Oysters sold in Freetown are mostly collected at the outlet of various streams into the sea, especially at Congo and Ascension Towns, where the Congo and Alligator Brooks respectively discharge, and also at the estuary of Granville Brook at Cline Town. We visited the oyster-collecting grounds at Congo and Granville Brooks and came to the conclusion that the former was the chief source of supply ; the villages of Lumley and Aberdeen also yield oysters, but they appear to be collected mainly for local use and were not investigated.

Oysters are gathered, chiefly by the women, at low tide, when they are chipped off the rocks, considerable care being taken not to break the shell and thereby lose the ' liquor.'

Oysters are sold in the market in two forms: either fresh or in the dried state. Probably more oysters are eaten fresh than dried, but, owing to the rapid deterioration of the fresh shell-fish, the dried form is more commonly met with in the markets. Dried oysters may be dismissed from suspicion as disease vectors because they require prolonged boiling to render them tender

enough for consumption. When required for food the fresh oysters are either split open with a knife or heated until the shells gape. The extracted oyster may be cooked in a very large variety of ways, stewing and frying being the most popular ; and it is note-worthy that in the latter case it is essential only to fry them for a short period, otherwise the flavour is lost.

As regards the eating of raw oysters, we have been unable to obtain any trustworthy evidence either for or against. Unquestionably the well-to-do creole class sometimes eats them thus, usually in the form of an oyster salad ; but generally speaking the eating of raw oysters amongst the native population in Freetown is looked upon as ' bad form,' and inquiry on this point generally elicited a somewhat indignant denial. We are of the opinion, however, that it is a common practice amongst certain classes to eat at least part of the catch uncooked.

We have been impressed with the part which the oyster plays in the native dietary in Freetown. Amongst the working class, at any rate, it is partaken of as a dish at least once a week throughout the year, and probably on an average very much oftener. This fact must be borne in mind when considering the apparent association between oyster-eating and the eight cases of enteric infection already recorded.

The oysters for bacteriological analysis were collected from the Congo Brook about half a mile up-stream from Congo Bridge, at points which we had previously ascertained to be the chief collecting grounds for the oysters supplied to the Freetown markets, and where, at the time when we were obtaining specimens, some native women were also busy collecting.

Site A was about 800 yards up-stream from Congo Bridge. Oysters were collected at low tide from rocks under water and at a few feet from the edge of the mangrove swamp. At this point the oysters are probably immersed in brackish water at low tide for a few hours, but for the rest of the time are covered with salt water.

Site B was about 200 yards up-stream from site A. This place represents the furthest point up-stream at which oysters are collected, and at low tide a fairly brisk flow of fresh water can be seen piling up against the incoming tide. It appears fairly certain, therefore, that shell-fish at this point must be immersed in brackish or even fresh water for some hours every day.

The technique adopted for the bacteriological investigation was to examine the individual oysters for the presence of *Bact. coli*, and, where found, to identify the strain in accordance with the classification used by the Ministry of Health in England, thus determining the incidence of faecal contamination amongst the oysters. In addition, corroborative evidence was sought by culturing for *Cl. welchii* and different species of streptococci. The results obtained are shown below in the Table.

Table showing the results of bacteriological examination of eight oysters obtained at sites A and B on the Congo River, four samples being examined from each site

Site	Oyster	Presence of				
		Faecal *Bact. coli*	Streptococci		*Cl. welchii*	Faecal contamination
			Faecal	Non-faecal		
A	1	Type I +	O	+	O	+
	2	Type II +	O	O	O	+
	3	O	O	O	O	O
	4	O	O	O	+	? +
B	5	Type I +	O	+	+	+
	6	Type I +	O	+	+	+
	7	Type II +	O	O	+	+
	8	? +*	O	O	O	? O

* Intermediate between *Bact. aerogenes* and *Bact. coli* Type II.

It will be seen from the above Table that no less than 5 out of the 8 oysters examined (62 per cent.) showed the evidence of faecal contamination, and it is of interest to note that the highest proportion of contaminated oysters was found amongst those collected furthest up-stream.

The above evidence does not of course prove that oysters were responsible for the few cases of enteric infection observed in Freetown at the beginning of this year ; but it provides positive proof that the local oyster is a dangerously contaminated foodstuff, and that, unless properly cooked, it is liable to act as a vehicle of intestinal infection.

THE ASSOCIATION OF BACTERIURIA WITH BLACKWATER FEVER IN WEST AFRICA

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I. INTRODUCTION

During recent years an extensive literature has accumulated regarding various possible factors in the aetiology of blackwater fever ; but, whereas publications on the biochemical changes in this disease have steadily increased, references to bacteriological investigations have on the contrary diminished, so that the comprehensive surveys of the literature given by Ross (1932) and by the reviews of Yorke in the *Tropical Diseases Bulletin*, extending over many years, record but few such investigations of the body fluids. This is probably due to the fact that there appears to be a steadily increasing consensus of opinion that blackwater fever is not due to a specific organism in addition to the malaria parasite ; nevertheless, until the cause of the disease is established, this opinion does not appear to us to be a sufficient reason for the present almost complete abandonment of bacteriological investigation. It is true that, in the comparatively few instances in which blood cultures have been carried out, the majority have proved sterile, a fact supported by injection experiments into human volunteers and into animals, such as those of Blacklock (1923), Thomson (1924), and that performed at the laboratories of the United Fruit Company (1926). It must, however, be remembered that the failure of these experiments is no certain proof that an organism may not be concerned in the aetiology of the disease, for, just as in the case of enteric, yellow fever and dengue, the bacterium or virus may only be demonstrable during a short period. Stephens and Christophers, so far back as 1900, recorded the presence of staphylococci in heart and spleen cultures made at an autopsy on a case of blackwater fever ; and Crichlow (1929) refers to the occurrence of ' staphylococcus-like ' organisms in the blood. As regards the urine in this disease, practically no bacteriological data are available. Ross (1932), in Southern Rhodesia, appears to have undertaken no cultural investigations of the blood or urine, but states that in the urine of blackwater cases ' Examination of the sediment for the presence of spirochaetes was invariably negative, but an interesting finding was that streptococci were present in the majority of the urines examined. Their significance is doubtful since they are also found in non-catheter specimens in other conditions with considerable frequency.'

The few blood cultures which we made in cases of blackwater fever have,

as in the case of other observers, proved sterile, but cultures of the urine from such cases have consistently yielded positive results, and we think that it may be of value to record them, although they fail to show any necessary connection between bacteriuria and blackwater fever.

Our attention was first drawn to the presence of bacteria in the urine of blackwater patients as far back as 1924, when, for the purpose of making certain observations on haemolysis, we wished to carry out experiments with sterile blackwater urine. For this purpose we obtained aseptically taken specimens of urine from a case of blackwater on the way to recovery, but on each occasion found that staphylococci were present. Under the impression that the organism was probably a contamination from the air or skin, we obtained a catheter specimen taken with all possible precautions ; this also yielded a pure culture of a staphylococcus.

In this paper we record the results of the examination of as many active and recovered cases of blackwater fever as we have recently encountered. Unfortunately, in so grave a disease it is essential to disturb the patient as little as possible, so that specimens taken with proper aseptic precautions were only obtained from 7 cases during or within a few days of the haemolysis. We also cultured the urines of 13 recovered cases, 12 of whom contracted their blackwater fever in West Africa, and most of whom gave a history of several years' freedom from the disease prior to this examination. For the purpose of control, the urines of 20 Europeans, who had been in this colony for two years or more, were examined, and also those of 24 West African natives, none of these controls being under treatment for any genito-urinary infection. No anaerobic cultures were carried out, although we are of the opinion that this line of investigation might yield interesting results.

The method adopted to obtain specimens of urine from the male as aseptically as possible was as follows :—The glans and meatus were carefully washed up with sterile soap and water, followed by a wash with absolute alcohol, and, having discarded the first flow of urine, specimens were collected directly into two or more broth tubes. We are of the opinion that this method is generally more satisfactory than the passing of a catheter, and it was usually adopted ; but in the case of females, natives and some patients in the active stage of blackwater, it was found necessary to pass a catheter after the preliminary washing-up just described. At the beginning of this investigation, primary cultures were obtained by taking samples of about 5 c.cm. urine into 20 c.cm. liver broth ; but later, nutrient broth was substituted, as it was found that the majority of the various organisms grew with equal regularity on this medium. The centrifuged deposit was never employed for cultural investigation. The broth and urine mixture was kept at 37° C. for four days, at the end of which time, if no growth was observed, sterility was confirmed by plating on nutrient agar. The usual bacteriological technique was followed for the isolation and identification of the organisms, and the results obtained are recorded in the Appendix. When

colony-counts were required, 2 c.cm. of the urine were sown in melted nutrient agar in Roux's bottles. In some of the early cases and in some of those obtained when travelling up-country, and also in the case of certain strains which died out early, it was not found possible to make a full study of the morphology, type of growth, and biochemical reactions of the organisms. Except in the few cases noted later, the bacteria isolated were, in our opinion, derived directly from the patient's genito-urinary tract (although we are unable to say from what part), for control exposures of the media under conditions similar to those existing at the time of collecting the specimen of urine never resulted in the isolation of cocci, although moulds and large spore-bearing bacilli were occasionally recorded. Further, frequent re-examinations of one of the two Europeans whose urine proved sterile on culture, using precisely the same technique as in other cases, invariably gave negative results, whereas many of the cases who proved to have a bacteriuria were re-examined and the same organism again isolated. In addition to the cultures, fresh and stained smears of the centrifuged deposit were examined in each case, and it was noted that, whereas bacilli were easily detected in fresh preparations, it was necessary to examine stained smears to recognize the presence of cocci.

II. THE RESULTS OF CULTURING THE URINE FROM SEVEN CASES OF ACTIVE BLACKWATER FEVER

All specimens were taken with the aseptic precautions already described, and no further reference will be made to this fact.

Case 1 (*no.* 1*a**). Syrian, male, aged 17 years ; catheter specimen obtained 24 hours after the commencement of his attack in 1924. Gram-positive diplococci were present in the immediately centrifuged urine, and 0·75 c.cm. dropped on to an agar-plate yielded three colonies. A pure culture of a staphylococcus was obtained on all media inoculated. This case reported 7 years later (see Case 12), and stated that he had had no subsequent attack of blackwater.

Case 2 (*no.* 2). Swiss, male, aged 22 ; catheter specimen obtained in 1924, some 7 days after the cessation of his haemoglobinuria. A staphylococcus was isolated in pure culture. A few drops sown on agar-plates yielded only a single staphylococcus colony.

Case 3 (*no.* 3). African, male, aged 7 ; blackwater fever in 1930. From catheter specimens obtained on two occasions during the attack, pure cultures of a staphylococcus were isolated in each instance, and films made from the immediately centrifuged urine showed the presence of this organism.

Case 4 (*nos.* 4*a and* 4*b*). West Indian native, male, aged 35 ; blackwater fever in 1931. Catheter specimen obtained during the attack yielded pure cultures of staphylococci. The centrifuged deposit in this case, examined immediately, showed, in addition to the usual blackwater débris, such large masses of this organism as to form a considerable part of the sediment.

Case 5 (*no.* 5). European, male ; admitted to hospital in Liverpool in 1932 with blackwater fever. A mid-stream specimen was obtained for us by Dr. F. Murgatroyd, who submitted the cultures to the hospital Pathologist ; a staphylococcus was isolated in pure culture.

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*The number quoted in brackets after the case-number refers to that under which the organism is described in the Appendix.

The remaining two active blackwater cases examined by us occurred at the end of our series. In order to estimate accurately the bacterial concentration in the urine of these cases, a somewhat more elaborate technique was adopted, and these cases will be described in some detail.

Case 6 (*nos.* 6*a*, 6*b*, 6*c and* 6*d*). Syrian, female, aged 28 ; blackwater fever in July, 1935. The patient was under treatment for quartan malaria, and haemoglobinuria first occurred on July 23rd ; when examined by us at her own house on July 24th, the haemoglobinuria, though still present, was diminishing. A considerable quantity of albumen was present, and the cellular deposit from the centrifuged urine was of the usual type associated with blackwater. In order to obtain as accurate colony-counts as possible, we adopted the following procedure : after the washing-up, a rubber catheter was passed and the urine collected in a series of five sterile test-tubes. As each tube was filled it was immediately placed on ice and subsequently taken to the laboratory, where the fourth and fifth specimens were inoculated into the various media within half an hour of being drawn off. The centrifuged deposit from tubes 4 and 5 showed a number of Gram-positive cocci and Gram-negative bacilli. Cultures made from the specimens yielded a staphylococcus, an enterococcus and a *Bact. coli*. The biochemical reactions, etc., of these organisms are recorded in the Appendix. Colony-counts, made after 24 hours' incubation, resulted in 317 organisms per c.cm. in specimen 4, and 335 organisms per c.cm. in specimen 5. Many fresh colonies developed after a further 24 hours' incubation, but their concentration was too great to allow of an exact estimation of their numbers. Owing to crowding, accurate counts were not made of the proportion of each species of organism present, but it was possible to distinguish most of the colonies ; and, whereas the *Bact. coli* and staphylococcus colonies occurred in about equal numbers, the enterococcal colonies were not so numerous. Further observations were not made on this case as the patient died two days later.

Case 7 (*nos.* 7*a*, 7*b and* 7*c*). European, male, aged 54 ; blackwater fever in August, 1935. His haemoglobinuria commenced during the night of August 14th, and he was examined by us in his own house on August 15th, 16th, 18th and 20th.

First examination, August 15th. The patient's condition prevented the collection of aseptically taken specimens, but the centrifuged deposit of recently passed urine showed the presence of large numbers of cocci, and 2 c.cm. sown in agar yielded a colony-count which was above 1,000.

Second examination, August 16th. The patient was washed up, employing the usual technique, and two mid-stream samples were collected ; these were placed on ice, taken to the laboratory and cultured within 20 minutes of passing. Samples of 2 c.cm. and 0·75 c.cm. uncentrifuged urine sown in melted agar yielded an average of more than 1,000 colonies per c.cm. urine. Eight c.cm. urine were sown in broth, and a pure growth of staphylococci was obtained.

Third examination, August 18th. On this date the condition of the patient had greatly improved and the urine was now free of haemoglobin. Only 15 c.cm. of the aseptically collected urine was available ; this was stored on ice, taken to the laboratory and cultured immediately. As on the previous occasion, a broth culture, consisting of 8 c.cm. urine and 20 c.cm. broth, yielded only staphylococci. A colony-count made on this date showed 1,300 organisms per c.cm. passed.

Fourth examination, August 20th. Five mid-stream samples were collected and the last two samples used for inoculating media. From both samples staphylococci were isolated in pure culture. In spite of the fact that large numbers of cocci were present in the centrifuged deposit, the number of colonies resulting from the sowing of the two samples in melted agar was surprisingly small, an average of 42 organisms per c.cm. being recorded. There would appear to have been some substance inimical to the growth of staphylococci in the urine on this date, for daily subculturing of the original broth media showed a steadily diminishing number of colonies, so that by the fifth day the broth and urine mixture was sterile in both the samples.

III. THE RESULTS OF CULTURING THE URINE FROM THIRTEEN RECOVERED CASES OF BLACKWATER FEVER

Case 8 (*no.* 8). European, male, aged 36 ; blackwater fever in 1926. Examination of his urine in 1930 showed the presence of staphylococci in pure culture, the organisms being sufficiently numerous to be demonstrable by immediate centrifuging of the urine.

Case 9 (*no.* 9). Swiss, male, aged 44 ; blackwater fever in 1928. Specimen of urine obtained in 1930. The centrifuged deposit examined within a few minutes of passing showed staphylococci, and cultures gave a pure growth of the same organism. In June, 1932, this patient had another attack of blackwater fever, at which time centrifuged specimens of his urine obtained during the haemoglobinuria (but without any aseptic precautions) showed the presence of staphylococci, together with a large spore-bearing bacillus of the air-borne type. It was unfortunate that the severity of this second attack, and the rapid evacuation of the patient to England, rendered it impossible to repeat our previous observation as to the presence of staphylococci in urine obtained with aseptic precautions.

Case 10 (*nos.* 10*a and* 10*b*). Moroccan, male, aged 34 ; blackwater fever in 1925 and again in 1927. Examined in January, 1933 ; this patient's urethra was in a somewhat damaged condition, owing to a long history of gonorrhoea. As in the previous cases, the urine yielded a growth of staphylococci, but in this instance a streptothrix was also isolated, together with a large spore-bearing bacillus which was regarded as a contamination.

Case 11 (*nos.* 11*a and* 11*b*). African, female, aged 9 ; blackwater fever in 1929. In this case a staphylococcus was isolated on examination of the urine in 1933, together with a streptococcus and a large Gram-positive bacillus.

Case 12 (*nos.* 12*a and* 12*b*). A re-examination in August, 1932, of Case 1 showed that this patient was still passing staphylococci in pure culture. Sixteen colonies resulted from sowing approximately 0·75 c.cm. urine on the surface of an agar-plate, and cocci were present in the immediately centrifuged urine.

Case 13 (*no.* 13). European, male, aged 35 ; blackwater in 1922. Specimen of urine obtained in 1924 yielded a staphylococcus in pure culture. Three drops on agar-slopes resulted in four colonies.

The following five cases were examined by one of us while in Nigeria. Several of the cases were investigated while travelling up-country, and in some instances the organism obtained from the urine died before its biochemical reactions could be properly tested.

Case 14 (*no.* 14). Syrian, male, aged 35 ; blackwater fever in September, 1931. A specimen of urine was obtained, one month after the attack, from which a pure culture of a staphylococcus was isolated. Two samples of 1 c.cm. each in broth yielded growth, in addition to the cultures made with larger quantities of urine.

Case 15 (*nos.* 15*a* and 15*b*). Syrian, male, aged 25 ; blackwater fever in 1925. On examination in 1931 the immediately centrifuged urine contained very numerous cocci, and inoculation of quantities of 1 c.cm. uncentrifuged urine, in addition to the routine larger quantities, yielded a staphylococcus in pure culture.

Case 16 (*no.* 16). European, male, aged 45 ; blackwater fever in 1909 and again in 1911 ; examined in 1931. From this case a long-chained streptococcus was isolated in pure culture.

Case 17. European, male, aged 34 ; blackwater fever in 1929 ; examined in 1931. Cultures remained sterile. This patient has recently been treated for gonorrhoea by irrigation *via* the urethra.

Case 18 (*nos.* 18*a and* 18*b*). European, male, aged 50 ; blackwater fever in 1923. A specimen of urine obtained in 1931 showed the presence of numerous staphylococci in the centrifuged deposit, and on culture a pure growth of this organism was obtained.

Case 19 (*nos.* 19*a and* 19*b*). European, male, aged 45 ; first attack of blackwater fever in 1928, followed by a similar attack in June, 1929. On examining this patient in 1935 he was found to be passing staphylococci and streptococci in his urine. A sample of urine,

obtained with the usual aseptic technique and sown in agar within 10 minutes of collection, yielded a colony-count of over 900 organisms per c.cm. of urine. The two species of organisms occurred in about equal numbers.

Case 20 (*no.* 20). This case is of some interest. Dr. C. B. Bamford (1934) has recorded in the *British Medical Journal* the case of a patient in England who, while under treatment with therapeutic malaria, developed an attack of haemoglobinuria. Our interest was aroused, and we wrote from Africa to Dr. Bamford, who kindly procured and cultured an aseptically taken specimen of urine from the patient, the culture being obtained 11 months after the attack of blackwater fever. Dr. Bamford informs us that the culture showed a growth of a staphylococcus.

It can be seen from these records that, in an examination of 20 active and recovered cases of blackwater fever, all but one had a bacteriuria. A staphylococcus was isolated in 18 instances, 14 times in pure culture and on 4 occasions in association with other organisms. The remaining positive case yielded a streptococcus in pure culture.

IV. THE RESULTS OF EXAMINING THE URINES OF TWENTY EUROPEAN AND TWENTY-FOUR NATIVE CONTROLS

All the European controls were selected from persons who had been at least two years in Sierra Leone—the majority had been there more than five ; none was known to be suffering from any genito-urinary infection. For the sake of brevity the results of the examination of the 44 controls have been recorded in tabular form (Table I).

It can be seen from these figures that of the 20 European controls examined, 18 (90 per cent.) were found to have a bacteriuria ; similarly, 16 (67 per cent.) of the 24 African controls were passing organisms in their urine. Out of a total of 44 controls, therefore, investigated by our methods of culture, 34 (77 per cent.) were found to be passing organisms in their urine at the time they were examined.

These figures would suggest a very high incidence of bacteriuria in West Africa, but we have little knowledge of how they compare with the incidence in other parts of the tropics or in more temperate zones. Surprising as it may seem, we have been able to find very few references to similar examinations carried out in other countries, the large literature concerning bacteriuria being almost wholly devoted to the bacteriology of pathological conditions of the genito-urinary tract. Dodds (1931) states that, in a series of examinations carried out in England, of 793 urines of unselected pregnant and puerperal cases, 11 per cent. contained organisms ; these results were arrived at by culturing both the uncentrifuged urine and the deposit after centrifuging. This writer also quotes the results obtained by several other workers who had examined by various methods the urines of pregnant women and had recorded figures varying from 8 per cent. to 80 per cent., the incidence of bacteriuria, according to the majority, being over 50 per cent.

We have already demonstrated the remarkable frequency with which

TABLE I

The results of examining the urine of 20 European and 24 native controls

Case no.	Nationality	Reference no. in Appendix	Condition at time of examination	Organisms isolated		
				Staphylococci	Streptococci	Other organism
21	European	21 a & b	Malaria	+	O	O
22	,,	22 a & b	,,	+	O	O
23	,,	23 a & b	Anaemia	O	O	+
24	,,	24	Normal	+	O	O
25	,,	25	,,	+	O	O
26	,,	26 a & b	Malaria	+	+	O
27	,,	27 a & b	Furunculosis	+	+	O
28	,,	28 a & b	Malaria	+	+	O
29	,,	29	,,	O	O	+
30	,,	30 a & b	Furunculosis	+	O	+
31	,,	31 a & b	Malaria	+	+	O
32	,,		Normal	O	O	O
33	,,	33 a & b	,,	+	O	O
34	,,		,,	O	O	O
35	,,	35	Malaria	+	O	O
36	,,	36	,,	+	O	O
37	,,	37	Normal	+	O	O
38	,,	38	,, *	+	O	O
39	,,	39	,,	+	O	O
40	,,	40	Malaria	+	O	O
41	African	41 a & b	Fracture	+	O	O
42	,,	42	,,	O	O	+
43	,,		Ulcer	O	O	O
44	,,	44	Epithelioma	O	+	O
45	,,		Hernia	O	O	O
46	,,	46	,,	+	O	O
47	,,	47	Hemiparesis	+	O	O
48	,,	48 a, b & c	Dislocation	+	+	+
49	,,	49 a & b	Appendicitis	+	O	O
50	,,	50 a, b & c	Hernia	+	+	O
51	,,	51 a & b	Elephantiasis	+	O	O
52	,,	52 a & b	Hydrocele	+	O	+
53	,,	53 a & b	Hernia & Hydrocele	+	O	O
54	,,		Elephantiasis	O	O	O
55	,,		Fracture	O	O	O
56	,,		Hernia	O	O	O
57	,,	57	Wound	+	O	O
58	,,	58	Elephantiasis	+	O	O
59	,,		Hydrocele	O	O	O
60	,,	60	Hernia	+	O	O
61	,,	61	Fracture	+	O	O
62	,,	62	Wound	+	O	O
63	,,		,,	O	O	O
64	,,		Hernia & Hydrocele	O	O	O

* Reference in text, page 447

bacteriuria is present in active and recovered cases of blackwater fever—95 per cent. positive amongst 20 cases examined. It is, however, impossible from such a small series of cases to infer that bacteriuria has necessarily any causal relationship with blackwater fever, especially since amongst 44 controls no less than 77 per cent. were also excreting organisms in their urine.

V. THE CONCENTRATION, PATHOGENICITY AND SOURCE OF THE ORGANISMS ISOLATED FROM ACTIVE AND RECOVERED CASES OF BLACKWATER FEVER AND FROM CONTROL CASES

We have already discussed the frequency of bacteriuria in Sierra Leone, and it appears advisable at this point also to consider briefly the concentration, pathogenicity and source of the organisms isolated.

1. *The concentration of bacteria in the urines of the cases examined.* In temperate zones the common occurrence of organisms in the urine is well recognized, but, unless they are present in large numbers or can be associated with a pathological condition in the patient, their presence is not usually regarded as significant. This view is in agreement with the reports of the Medical Research Council (1929), which state that staphylococci are constantly present in the anterior urethra.

Unfortunately, we did not fully investigate this question of the concentration of bacteria in the early part of our work, so that most of our observations on the bacteria occurring in active and recovered cases of blackwater fever, as well as in control cases, were qualitative rather than quantitative. Certain observations, however, were carried out in the early cases, which consisted of an examination of the centrifuged deposit and, in some instances, of an estimation of the number of colonies resulting from sowing a few drops of the uncentrifuged urine on the surface of agar-plates. Later, two active and one recovered case of blackwater fever came under our observation and were examined as regards the concentration of bacteria in their urine, with the results already quoted. At the same time we examined a small series of controls consisting of Europeans and Africans, who were known to be excreting organisms in their urine and who were chosen as being as near normal as possible. The colony-counts in these cases were made on 2 c.cm. of urine which were sown in agar immediately after collection. The five European controls yielded colony-counts respectively of 126, 20, 3, 1 and less than 1 bacterium in the 2 c.cm. urine plated. The five African controls yielded colony-counts respectively of 14, 8, 2, 1 and less than 1 bacterium in the same quantity of urine.

One of the two active blackwater cases (Case no. 6) examined by this technique yielded a colony-count of over 600 organisms in the 2 c.cm. examined ; while the recovered case (Case no. 18) gave a count of over 1,800 organisms—results far higher than any count noted in the controls. The only other active blackwater case in which accurate colony-counts were attempted was examined both during and after the haemoglobinuria. During the haemoglobinuria the colony-counts exceeded 1,000 organisms for each c.cm. of urine examined ; a

similar result was recorded on the day on which the haemoglobinuria ceased, but two days later only 42 organisms per c.cm. were noted. Of the remaining 17 active and recovered blackwater cases, in 10 instances some idea of the number of bacteria in the urine was formed either by culture (in 4 cases) or by examining the centrifuged deposit from the freshly passed urine (in 6 additional cases). The method adopted in the former 4 cases, i.e., the sowing of a few drops of the urine on the surface of an agar-plate, did not allow of any very accurate enumeration of the organisms, but, from the figures already quoted, the concentration of the bacteria probably varied in different cases from 4 to 20 per c.cm. approximately. These counts seem low in comparison with the high figures just cited for the three accurately counted cases at the end of our series, a difference due to the fact that in the early part of the investigation when organisms were numerous in the centrifuged deposit only the usual routine cultures intended for the identification of the organisms were made. These figures, therefore, probably represent the lowest counts which would have been obtained in the series. In the 6 latter cases, in addition to obtaining positive cultures, bacteria were found in the centrifuged deposit, in some cases in large numbers. In the 44 Europeans and African controls, although 77 per cent. yielded positive results by culture, organisms were rarely observed in the centrifuged deposit and, when present, were usually but few in number.

We have already proved that the incidence of bacteriuria is higher amongst active and recovered cases of blackwater fever than it is amongst control cases. The observations just recorded appear to suggest that the concentration of bacteria is also higher in the former cases.

2. *The pathogenicity of the bacteria isolated from the urines examined.* It will be noted that no reference has so far been made to tests of the pathogenicity of the organisms. At the time of the investigation an epidemic was in progress amongst our experimental animals, and it was thought advisable to test in England the pathogenicity of a number of the strains which had been isolated from active blackwater cases, recovered cases and controls. When inoculated into experimental animals these strains were found to be almost non-pathogenic ; this, however, may have been due to frequent subculturing, since two strains, which had been tested in West Africa and found to be pathogenic, had lost their virulence when retested in England.

As already explained, our controls were selected from persons showing no genito-urinary infection and, in the great majority of instances, no pyrexia. With one exception, amongst the control European and African cases no signs were observed which could be associated with the bacteriuria. The one exception was Case no. 38, a European who had been selected as a healthy normal control, and who had not suffered from malaria for over a year. His urine, collected with the usual aseptic non-catheter technique, yielded a pure growth of staphylococci in such numbers that accurate counts were not made, and it can only be stated that the count considerably exceeded 1,000 colonies in the 2 c.cm. cultured.

On the morning following the collection of the sample the patient experienced a rigor, and for 24 hours sustained a temperature of 100°–102° F.; examination of his blood, etc., revealed no cause for his illness other than the bacteriuria. Even amongst the recovered blackwater cases, where the bacterial concentration was frequently very high, we observed no symptoms which could be referred to the bacteriuria. Finally, as is shown later in the text, the organisms isolated from active and recovered cases of blackwater fever did not markedly differ either in their morphological, cultural or biochemical characters.

We can produce no evidence, therefore, that the organisms isolated were pathogenic.

3. *The source of the bacteria isolated from the urines examined.* The fact that bacteriuria was equally common in urines obtained by catheter and in those obtained after the usual washing-up process, does not necessarily indicate that the source of the organisms was in the bladder or higher, since in the former instance they might have been carried up from the urethra by the catheter. As direct puncture of the bladder, by which the urethra is avoided, was inexpedient, we tried to obtain information as to the source of the bacteria by culturing urine obtained by ureteral catheterization.* During the past few months it has been possible to examine 7 such specimens, of which 6 were obtained from Africans and 1 from a Syrian. In these cases only a small quantity of urine—about 1 c.cm.—was available for culture. One of these cases, an African, yielded a pure growth of a staphylococcus, while the remaining 6 proved sterile. In a control series of Africans, in which the urine was obtained from the bladder by catheter, culturing of 1 c.cm. urine proved sterile in 6 of the 9 cases examined, while 3 yielded growths of cocci. These figures show a lower incidence of bacteriuria when the urine is obtained from above the bladder, but no conclusions can be based on such a small series. Since no ureteral catheterizations were carried out on any of the active or recovered blackwater cases, we are unable to state the source of the high bacterial concentrations recorded in the majority.

It is difficult to define what concentration of the bacteria isolated from the urines examined should be regarded as possibly derived from the urethra. None of the control European or African cases yielded a count sufficiently high to exclude this possibility. We are, however, convinced that the very high bacterial concentrations observed in some of the active and recovered cases of blackwater fever could not be accounted for in such a way.

VI. TYPES OF ORGANISMS ISOLATED

Details regarding the biochemical reactions of the organisms isolated are given in the Appendix, and we propose to deal here only with such points as

*Aseptically tapping the bladder of both laboratory animals and wild rats in Freetown has proved to us that staphylococci in pure culture are sometimes present in the urine of these animals before it has reached the urethra.

are not there referred to. In addition to the data in the Appendix, records were kept of the type of growth on liquid and solid media at, respectively, 1 and 5 days' interval after inoculation. Each organism had its morphology studied at the same periods by means of Gram's stain and carbol thionin blue. Pigment-formation was looked for on agar-slope cultures at bench temperature during a period of three weeks. In the case of the staphylococci, a typical *aureus* pigment was never observed, the organism being always of the *albus* type, although at the same time it was noted that staphylococci of the *aureus* type were sometimes present in cultures made from skin lesions of the same cases. Certain other tests were in every instance carried out, but are not recorded in the Appendix since they invariably resulted in a negative finding ; these included the following : (*a*) indol formation, (*b*) ammonia production, (*c*) presence of growth in protein-free citrate broth, (*d*) splitting of urea, as indicated by changes in the pH of a urea peptone culture. There were two exceptions to these negative findings : indol was produced by the *Bact. coli* isolated from Case no. 6, and a strong ammonia reaction was given by the staphylococcus isolated from the urine of Case no. 56. The failure to observe urea-splitting is of interest, since various authors, amongst them Earlam (1930), Hellström (1930) and Sas and Szold (1931), have shown that a large proportion of staphylococci isolated from the urine of patients suffering from genito-urinary lithiasis possess the property of splitting urea, and these cocci are regarded by them as playing a part in the production of stone, a disease which is rare in West Africa. It is possible that our failure to demonstrate urea-splitting was due to the use of too often sub-cultured strains, a point mentioned by Laidley (1930).

The streptococci isolated were subjected to the same form of examination as the other bacteria, but since it is generally recognized that their classification largely rests on their power of haemolysis, and since this was not investigated, it is not possible to classify them into species. As judged by their power of surviving heating to 60° C. for half an hour and by their sugar reactions, some of them were enterococci.

We believe that we are correct in saying that at the present time it is almost impossible to differentiate the various species and subspecies of staphylococci. It will be remembered that 5 of the 7 active cases of blackwater fever yielded a pure culture of staphylococcus. On careful examination of these strains, however, it was found that, not only did they differ in the different cases, but also more than one strain (as judged by morphological and cultural characteristics, as well as by biochemical reactions) was sometimes isolated from a single individual. How far these variations can be relied upon as a means of differentiating one strain from another we are not prepared to state, but we can say that cocci giving similar morphological, cultural and biochemical reactions were also present amongst controls with no history of blackwater fever.

During the early part of the investigation it was noted that staphylococci isolated from different sources frequently showed a very diplococcal appearance,

so that at first we believed that we were dealing with an organism distinct from the staphylococcus group. In many cases this diplococcal character of the staphylococci was sufficiently marked to render difficult their morphological differentiation from a short-chained streptococcus, a characteristic, however, which was usually lost on repeated subculture. This formation was sometimes noted in cocci in the immediately centrifuged urine ; and it seems probable that, in the absence of cultural investigations, the organisms referred to by Ross (1932) as streptococci may have belonged to these streptococcal-looking staphylococci.

It will be seen from the Appendix that, of the 82 strains of organisms examined, 74 could be definitely classified, and of these 63 were *Staphylococci* (Table II), and 11 were *Streptococci* (Table III). The remaining 8 organisms have not, as yet, been finally classified, but, by means of their biochemical reactions (shown in the Appendix) and the morphological and other cultural characters (not here recorded), a tentative classification has been affixed to them, as is shown in Table IV.

VII. SUMMARY AND CONCLUSIONS

Our results and conclusions may be summarized briefly as follows :—

In 7 cases of blackwater fever examined during the active stage of the disease all 7 were found to be excreting organisms in their urine. Amongst so small a series of cases, such as association may, of course, be merely a coincidence ; we cannot, however, dismiss it as such, until a case of active blackwater fever is encountered in whose urine no organisms are to be found. We have not observed such a case in Sierra Leone, and the one case examined for us in England was also associated with a bacteriuria.

In 13 recovered cases, examined at various periods from one month to ten years after the attack, 12 were found to have bacteria in their urine, while in one, examined two years after the attack, no bacteria were found on culture.

Amongst 44 European and African controls, examined by the same technique, 34 were found to be similarly excreting organisms. The incidence of bacteriuria, therefore, amongst the active and recovered blackwater cases was higher than amongst the controls, 95 per cent. as compared with 77 per cent.

In the small series of active and recovered blackwater cases in which quantitative estimations were made, the concentration of bacteria was generally found to be higher than amongst the control cases.

A comparison of organisms of the same genera, i.e., staphylococci or streptococci, isolated from active and recovered blackwater cases and from control cases, showed no essential differences in morphological, cultural or biochemical characters.

We have been unable to produce any evidence that bacteria isolated from the urines of active and recovered blackwater cases, or from control cases, show any marked pathogenicity.

Although it has thus been shown that blackwater fever is constantly associated with bacteriuria in the series of cases examined by us, and that the concentration of bacteria is usually high, yet, since we have been unable to demonstrate any marked pathogenicity in the organisms isolated, and since controls show that bacteriuria is widespread amongst Europeans and Africans in Sierra Leone, it follows that its mere presence in cases of blackwater fever cannot be regarded as evidence that it bears any causal relationship to the disease. Further, the results obtained prove that a combination of chronic malaria undergoing treatment with quinine and associated with bacteriuria does not necessarily result in blackwater fever, for several of our European control cases presented this combination, although in these cases the concentration of bacteria was low. In addition, some of the controls, who at the time of examination were similarly excreting a small number of bacteria in their urine but were not suffering from malaria, subsequently contracted malaria without developing haemoglobinuria.

The remarkedly high bacterial concentration which we have recorded in some of our active and recovered blackwater cases is the only feature which we have not observed in the series of control cases. Whether the high bacterial concentration in the urine in these cases was a mere coincidence, or whether it played any part in the causation of the disease, cannot be determined without examining a larger number of cases.

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APPENDIX

Appendix showing the biochemical reactions of the 59 *Staphylococci*, 11 *Streptococci* and 8 incompletely identified organisms isolated.

In the sugar-fermentation results :—A = acid ; AG = acid and gas ; ACBD = acid, clot, bleaching and digest ; lt = late ; NC = no change during 3 weeks' observation ; and the numeral after the reaction indicates the day subsequent to the inoculation on which the change was noted. Thus ' A3 wk ' signifies a weak acid reaction on the third day. The sign + = positive ; O = negative ; and — = not examined.

TABLE II

Showing the biochemical reactions of the *Staphylococci* isolated

	No.	Dextrose	Maltose	Mannite	Lactose	Sucrose	Salicin	Dextrin	Galactose	Dulcite	Gelatine liquefaction	Litmus milk	Reductase	Catalase	Hydrogen sulphide	Nitrate reduction	Methyl red	Vosges Proskauer
Active blackwater cases	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	2	A	—	NC	NC	A	—	A	—	—	—	—	—	—	—	—	—	—
	3	A	A	A lt	A lt	A	NC	A	A	NC	+	ACBD	+ wk	+	+	+ wk	+	+
	4a	A1	A2	A8	NC	A3	NC	NC	A2	—	O	O	O	+	+	O	O	O
	4b	A1	A1	A2	A3	A1	A2	NC	A1	NC	O	ACBD	+	+	+	+	+	O
	6b	A1	A2	NC	A2	A2	NC	A8 wk	A3	NC	O	ACB	O	O	+	+	O	O
	6d	A1	A1	NC	A3	A1	NC	NC	A3	NC	O	AB	O	O	+ wk	+	O	O
	7a	A3	A2	NC	A3	A2	NC	NC	A2 wk	NC	O	A wk	O	+	+	+	O	O
	7b	A3	A3	NC	A3	A3	NC	NC	A3	NC	O	ACBD	O	+	+	+	O	O
	7c	NC	NC	NC	NC	A4 wk	NC	NC	NC	NC	O	A	O	+	+	O	O	O
Recovered blackwater cases	8	A	A	NC	A lt	A	NC	A	A	—	O	ACBD	+	+	+	+	+	O
	9	A	A	NC	A lt	A	NC	A	A	NC	O	ACBD	O	+	+	+	+	O
	10a	A1	A10	NC	A2	A1	NC	A5	A2	NC	+	ACBD	+ wk	+	O	+	+	+
	10	A1	A4	A4	A4	A1	NC	NC	A4	NC	+	ACB	O	+	+	+	O	O
	11a	A1	A3	NC	A3	A3	NC	A10	A1	NC	+	ACBD	O	+	O	+	+	+
	12a	A1	A2	A5	A2	A3	NC	NC	A2	—	O	B	+ wk	+	+	+	+ wk	O
	12b	A1	A2	A2	A2	A2	NC	NC	A2	—	O	B	+ wk	+	+	O	+	O
	13	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	14	A	A	A	A	NC	A	—	—	NC	+	ACBD	—	—	+	O	+	O
	15a	A	A	A	NC	A	NC	—	—	NC	O	O	—	—	+	+	+	O
	15b	A	A	A	A	A	NC	—	—	NC	+	AC	—	—	+	+	O	O
	18a	A	A	NC	NC	A	NC	—	—	NC	O	O	—	—	O	O	O	O
	18b	A	A	NC	A	A	NC	—	—	NC	O	A	—	—	+	+	O	O
	19a	NC	A3	NC	NC	NC	NC	NC	NC	NC	O	O	O	+	+	O	O	O
	20	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

European controls	21a	A1	A1	A5	A2	A2	NC	NC	A1	NC	O	O	O	+	+	+	+ wk	O
	21b	A2	A2	A4	A3	A2	NC	NC	A2	NC	+	ACBD	O	+	+	+	O	O
	22a	A2	A2	NC	A2	A12	NC	A3 wk	A2	NC	+	ACBD	O	+	+	+	+	O
	22b	A1	A1	NC	A2	A2	NC	A6 wk	A1	NC	O	ACBD	O	+	+	+	+	O
	24	A	A	NC	A lt	A	NC	A	A	NC	O	ACBD	+	+	+	+	+	O
	25	A	A	NC	A lt	A	NC	A	A	NC	O	ACBD	+	+	+	+	+	O
	26	A3	A2	A5 wk	A2	A2	NC	NC	A2	—	O	ACB	O	+	+	+	+	O
	27	A1	A2	NC	A3	A2	NC	A12	A4	—	O	ACBD	+ wk	+	+	O	+	+ wk
	28	A1	A1	A3	A1	A1	NC	A1	A1	NC	O	AB	+ wk	+	+ wk	+	+	O
	30a	A1	A1	A3	A2	A1	NC	NC	A1	NC	O	A	O	+	+ wk	+	+	O
	31a	A1	A2	NC	A2	A1	NC	A8 wk	A1	NC	O	ACBD	O	+	+ wk	+	+	O
	33a	A1	A1	A5	A1	A1	NC	NC	A1	NC	O	A	O	+	+	+	+	+ wk
	33b	A2 wk	NC	NC	NC	NC	NC	NC	NC	NC	O	O	O	+	—	O	O	O
	35	A1	A2	NC	A2	A2	NC	NC	A3	NC	O	ACB	O	O	O	O	O	O
	36	A3	NC	NC	A4	A5	NC	NC	A3	NC	+	A	O	O	O	+	O	O
	37	A3	A8 wk	A8 wk	NC	NC	NC	NC	A3	NC	+	A	O	+	+	+	O	O
	38	A1	A1	A3	A1	A1	NC	A7	A1	NC	O	A	O	O	O	+	+ wk	O
	39	A1	A2	NC	NC	A2	NC	NC	NC	NC	+	O	O	+	+	+	O	O
	40	A1	A2	NC	A1	A1	NC	NC	A4	NC	+	ACBD	O	+	+ wk	+	O	O
African controls	41a	A1	A1	A6	NC	A5	NC	A6	A1 wk	NC	O	O	O	+	+	+	+ wk	+
	41b	A1	A3	NC	A1	A1	NC	A6	A1	NC	O	A	O	+	O	+	+	O
	46	A1	A2	A10	A5	A2	NC	A5	A2	NC	O	ABC	O	+	O	O	O	O
	47	A2	A2	NC	A2	A2	NC	A4	A2	NC	+	ACBD	O	+	+ wk	+ wk	O	O
	48	A1	A1	A3 wk	A2	A1	NC	NC	A2	NC	O	ACBD	O	+	O	O	O	O
	49a	A1	A3	A4	NC	A1	NC	A2	A4	NC	+	ACBD	O	+	+	+	O	O
	49b	A2	A1	NC	A4	A4 wk	NC	NC	A2	NC	+	ACBD	O	+	+	+	O	O
	50a	A1	A1	A6	A1	A1	NC	NC	A1	NC	O	ACBD	O	+	+	+	+	O
	50b	A1	A1	A4	A3	A2	NC	A9	A1	NC	O	A	O	+	+	O	+	O
	51a	A1	A1	A6	NC	A1	NC	NC	A1	NC	O	A	O	+	+	O	+	O
	51b	A1	A1	NC	A1	A1	NC	A5	A1	NC	O	ACB	O	+	+	+	+	O
	52a	A1	A1	A3	NC	A1	NC	A18	A1 wk	NC	O	B	O	+	+	+	+	O
	53a	A1	A1	A4	A1	A1	NC	NC	A1	NC	O	ACBD	+ wk	+	+	+	+	O
	53b	A1	A1	NC	A1	A1	NC	A9 wk	A1	NC	+	ACBD	O	+	+	+	+	O
	56	A1	NC	NC	A8	NC	NC	NC	A1	NC	+	ACBD	O	O	O	O	O	O
	58	A1	A1	NC	A1	A1	NC	NC	A1	NC	O	AB	+ wk	O	O	+	+	O
	60	A3	A2	NC	A4	NC	NC	NC	A7	NC	O	A	O	—	+	+	O	O
	61	A1	A1	NC	A1	A1	NC	NC	A2	NC	+	ACB	O	O	O	+	O	O
	62	A3	A1	NC	A5	A3	NC	NC	A2	NC	+	AB	O	O	O	+	O	O

TABLE III

Showing the biochemical reactions of the *Streptococci* isolated ; AB = active blackwater, RB = recovered blackwater, EC = European control, and AC = African control

	No.	Dextrose	Maltose	Mannite	Lactose	Sucrose	Salicin	Dextrin	Galactose	Dulcite	Gelatine liquefaction	Litmus milk	Reductase	Catalase	Hydrogen sulphide	Nitrate reduction	Methyl red	Vosges Proskauer	Heat resistance*
AB	6a	A1	A1	NC	A9 wk	A9 wk	A3	A3	A3 wk	NC	+	AB	O	O	+	O	O	O	+
RB	11b	A1	A1	A1	A3	A1	A1	A1	A1	NC	+	ACBD	O	O	O	O	O	O	+
	16	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	19b	A1	A1	A4	A1	NC	A1	A2	A1	NC	O	ACBD	O	O	+	O	O	O	+
EC	26b	A1	A1	A1	A13	A1	A1	A1	NC	—	O	ACBD	O	O	O	O	O	O	+
	27b	A1	A1	A1	A2	A2	A1	A1	A1	—	O	ACBD	O	O	O	O	O	O	+
	28b	A1	A1	A1	A1	A1	A1	A1	A1	NC	+	ACBD	O	O	O	O	O	O	O
	31b	A1	A1	A1	A1	A1	A1	A1	A1	NC	+	ACBD	O	O	O	O	O	O	+
AC	44	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	48a	A1	A2	NC	A2	A1	NC	A2	A2 wk	NC	O	AB	O	O	O	O	O	O	O
	50b	A1	A1	NC	A3	A1	NC	A2	A1	NC	O	AC	O	O	O	O	O	O	O

* '+' in this column indicates growth after heating for 30 minutes at 60° C.

TABLE IV

Showing the biochemical reactions of 8 organisms not completely identified

No.	Tentative classification	Dextrose	Maltose	Mannite	Lactose	Sucrose	Salicin	Dextrin	Galactose	Dulcite	Gelatine liquefaction	Litmus milk	Reductase	Catalase	Hydrogen sulphide	Nitrate reduction	Methyl red	Vosages Proskauer
6c	*Bact. coli*	AG1	AG2	AG1	AG1	NC	AG2	NC	AG1	AG3	O	ACB	O	O	+	+	+	O
23a	*Corynebacterium*	NC	NC	NC	NC	NC	NC	NC	NC	NC	O	O	O	+ wk	O	O	O	O
23b	*Streptococcus*	A5	A5	A6	A2	A2	NC	A3 wk	A2	NC	O	ACBD	O	O	+	O	+	O
29	*Streptococcus*	A4	NC	NC	NC	A3 wk	NC	NC	A3	NC	O	O	O	+	O	O.	O	O
30b	*Diplococcus crassus*	A1	A1	A1	A2	A1	A1	A1	A1	NC	O	ACB	O	O	+	O	O	O
42	*Neisseria*	A10	A15	NC	NC	A4	NC	NC	NC	NC	O	A	O	+	O	O	O	O
48c	*Corynebacterium*	NC	NC	NC	NC	NC	NC	NC	NC	NC	O	O	O	+	+	O	O	O
52b	*Corynebacterium*	NC	NC	NC	NC	NC	NC	NC	NC	NC	O	O	O	+	O	?+ wk	O	O

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ADDENDUM

Since the above paper was written we have had the opportunity of examining another case of blackwater fever and of obtaining specimens of urine collected aseptically during the active stage.

Mr. F. P., a European, male, age 38; blackwater fever in October, 1935. His haemoglobinuria commenced on the evening of October 8th, while he was in hospital undergoing treatment for malignant tertian malaria.

He was examined by us on October 11th, at which time the haemoglobinuria had almost ceased, but casts and the usual renal débris seen in cases of blackwater fever were still present. The attack was a mild one and did not incapacitate the patient, so that with his co-operation we were able to collect a series of samples taken with proper aseptic precautions. After the usual washing up with sterile soap and water, sterile water, and finally with alcohol, the penis was wrapped in a sterile towel and four mid-stream samples were collected. These samples were immediately placed in a thermos flask containing ice, taken to the laboratory and sown on various media within half an hour of collection, the second and third samples being used for cultural purposes.

Both samples yielded a pure growth of staphylococci. These strains have not yet been fully examined, but apparently they fall into the same category as some of those already recorded in our active blackwater series.

The colony-counts recorded from the samples collected at this stage of the haemoglobinuria (i.e., on the third day) were extremely high, no. 2 sample being estimated to contain 3,200 staphylococci per c.cm., and no. 3 sample, 3,100 cocci per c.cm. Owing to crowding both these counts can only be regarded as approximate, and probably the actual number of organisms was even greater.

SUSCEPTIBILITY AND RESISTANCE TO A TRYPANOSOME INFECTION

X.—SPECIFIC CHARACTER OF THE IMMUNITY PRODUCED IN RATS BY THE INJECTION OF SUSPENSIONS OF DEAD TRYPANOSOMES

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In the preceding paper in this series (Kligler and Comaroff, 1935), data were presented showing that the repeated injection of a suspension of dead trypanosomes into rats enhances their resistance to an infection with the homologous strain of the organism. It was of interest to determine whether this acquired resistance was specific. The experiments presented below deal with this aspect of the problem.

The procedure was the same as in the experiments referred to above. Rats were infected with the passage-strain of a given trypanosome. When the blood infection reached about 800,000 trypanosomes per c.mm., the rats were bled into a saline citrate solution. By fractional centrifugation it was possible to remove first the red cells and then the serum, and to obtain a heavy suspension of trypanosomes in saline, practically free of blood cells. This saline suspension was incubated for 2 hours at 37° C., and was then left at ice-box temperature until the trypanosomes were dead.

This saline suspension of dead trypanosomes was used for immunization. Each rat received five injections of 1·0 c.cm. of the suspension on 5 successive days. The infecting dose was given 3–5 days after the last injection.

Five series of experiments were carried out. In two of the series the rats were treated with *T. evansi*, and equal groups were infected with *T. evansi*, *T. gambiense* and *T. equi* respectively. In two of the series the rats were treated with *T. gambiense* and infected as above. In the last experiment the rats were treated with *T. equi* and infected with the 3 strains.

The results are summarized in the protocols and Tables below :

Series 1.—In the first series, 45 rats were given 5 injections of *T. evansi*. Three days after the last injection, these rats were divided into 3 groups, each of 15 rats, and each group was infected with a different species of trypanosome. There were corresponding untreated controls of 8 rats per group.

In the second series, the procedure was identical, except that there were only 10 rats in each group. The results are summarized in Table I.

TABLE I

Resistance of rats immunized with suspensions of *T. evansi* to infections with the homologous and heterologous species of trypanosomes

Experiment 1	No. of rats	Average weight	No. of injections	Infected with											
				T. evansi				*T. gambiense*				*T. equi*			
				Duration of infection : days				Duration of infection : days				Duration of infection : days			
				Min.	Median	Mean	Max.	Min.	Median	Mean	Max.	Min.	Median	Mean	Max.
Treated	15	55·0	5					5·5	6·5	7·2	10·5				
Control	8	52·5	0					5·5	7·0	7·3	12·5				
Treated	15	54·0	5	**8·5**	**12·5**	**12·5**	**19·5**								
Control	8	53·0	0	5·5	7·5	7·8	10·5								
Treated	15	55·0	5									8·5	14·5	13·8	17·5
Control	8	55·5	0									10·5	16·5	16·3	17·5
Experiment 2															
Treated	10	67·0	5					9·0	13·5	13·6	18·0				
Control	7	69·0	0					9·0	9·0	10·1	13·0				
Treated	10	67·0	5	**12·0**	**13·0**	**13·2**	**15·0**								
Control	7	66·0	0	6·0	7·0	7·3	12·0								
Treated	10	66·0	5									13·0	16·5	16·3	20·0
Control	7	70·0	0									15·0	16·0	17·0	21·0

TABLE II

Resistance of rats immunized with suspensions of *T. gambiense* to infections with the homologous and heterologous species of trypanosomes

Experiment 3	No. of rats	Average weight	No. of injections	Infected with											
				T. evansi				*T. gambiense*				*T. equi*			
				Duration of infection : days				Duration of infection : days				Duration of infection : days			
				Min.	Median	Mean	Max.	Min.	Median	Mean	Max.	Min.	Median	Mean	Max.
Treated	10	66·3	5					**14·0**	**16·0**	**15·8**	**18·0**				
Control	7	61·7	0					6·0	7·0	6·4	8·0				
Treated	10	62·0	5	6·0	8·0	8·0	11·0								
Control	7	59·7	0	6·0	8·0	7·6	9·0								
Treated	10	64·2	5									13·0	15·0	15·2	18·0
Control	7	59·0	0									15·0	18·0	16·6	19·0
Experiment 4															
Treated	9	67·8	5					**12·0**	**16·0**	**15·6**	**19·0**				
Control	7	63·1	0					7·0	11·0	10·6	13·0				
Treated	10	67·6	5	6·0	6·0	6·4	8·0								
Control	7	62·0	0	6·0	6·0	6·4	8·0								
Treated	10	66·8	5									10·0	11·0	11·5	14·0
Control	7	64·0	0									9·0	9·0	10·3	13·0

TABLE III

Resistance of rats immunized with suspensions of *T. equi* to infections with the homologous and heterologous species of trypanosomes

Experiment 5	No. of rats	Average weight	No. of injections	Infected with											
				T. evansi				*T. gambiense*				*T. equi*			
				Duration of infection : days				Duration of infection : days				Duration of infection : days			
				Min.	Median	Mean	Max.	Min.	Median	Mean	Max.	Min.	Median	Mean	Max.
Treated	8	50·7	5					5·0	5·0	5·5	7·0				
Control	5	50·6	0					5·0	6·0	6·0	7·0				
Treated	8	50·8	5	7·0	8·0	7·6	9·0								
Control	5	48·6	0	6·0	7·0	7·0	8·0								
Treated	8	51·0	5									18·0	22·0	22·5	32·0
Control	5	48·6	0									7·0	9·0	9·2	12·0

Series 2.—The identical procedure was used in experiments 3 and 4, except that suspensions of *T. gambiense* were used for immunizing purposes. The results are summarized in Table II.

Series 3.—In the last experiment *T. equi* was used as the immunizing strain ; otherwise the procedure was the same as in the preceding experiments. The results are given in Table III.

As can be seen from the data presented in the Tables, the results were quite consistent. The injection of dead trypanosomes enhances the resistance of the rats to a subsequent infection with the homologous strain. With the exception of the group of rats in experiment 2 which was infected with *T. gambiense*, the heterologous species affected the treated animals in the same manner as the untreated controls. In other words, the treatment did not increase the resistance of these rats to an infection with an heterologous organism as compared with untreated controls. It is apparent from these experiments that a partial immunity can be induced in rats by 5 successive injections of a suspension of dead organisms, and that this immunity is specific in character.

A number of interesting problems arise from these results. In view of the fact that this effect was produced by an injection of the rat-passage strain, it will be of interest to determine whether successive immunizations with a variety of strains derived from the same species, either by passage through other animals or from partially immune rats, will induce a more solid immunity. Assuming that the nature of the immunity is the same as in bacterial infections, the failure to obtain solid immunity is, presumably, attributable to the resultant selection of serum-resistant strains. If the lability or variability of strains is limited, it should be possible to obtain a polyvalent vaccine which will yield a solid immunity against a particular species. It is also of interest to note that the best results were obtained with *T. equi* which had only recently been passed into rats and had not as yet attained its maximum virulence for this animal. The question arises, therefore, whether the immunizing capacity is associated with virulence, or whether the relatively low grade of immunity produced is more effective against strains of low virulence.

It would seem that the procedure and results reported in this paper open the possibility of a more systematic study of active protozoan immunity than has hitherto been carried out.

SUMMARY

Rats treated with suspensions of dead trypanosomes develop an increased resistance to an infection with the homologous strain. This enhanced resistance is specific ; an infection with another species follows the same course as in control non-treated animals.

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NOTES ON BRAZILIAN MOSQUITOES: SPECIES OBSERVED IN THE AMAZON VALLEY, AND RECORD OF *AËDES ALBIFASCIATUS* MACQ. INVADING A SHIP IN HARBOUR

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During a number of voyages up the River Amazon in 1933 and 1934, one of us (G.R.W.) made observations on the mosquitoes, especially on the adults of *Sabethes* and other genera occurring in the forest. A collection of several species encountered was made, and some of the mosquitoes tended to differ in one or more particulars from the usual condition of the species as defined in recent monographs and keys. The majority of observations were made along a narrow forest-path at Utinga just outside Pará in September, 1934.

Mansonia arribalzagiae Theo.

This was the commonest and most troublesome mosquito in the Utinga forest, and was found in the dampest and most gloomy places.

In one ♂, agreeing externally with *M. arribalzagiae* as defined by Shannon (1931), the hypopygium was very similar in most respects to that illustrated by that author. The clasper, however, was without a definite expansion, unless this had contracted on both sides in such a way as to be invisible in whatever aspect the segment was viewed.

Seven other species of *Mansonia* were collected in the Amazon valley, most of the species agreeing exactly with characters given in recent keys and descriptions.

Mansonia amazonensis Theo.

The two complete ♀♀ taken differed from Dyar's (1928) description in having the hind tarsal rings very broad on the third and fourth segments, that on the fourth being more than half its length. Dyar describes all the tarsal rings as 'small,' but in the original description Theobald notes that the rings on the hind legs are much broader than the ones on the other legs.

M. titillans ; flavescent aberration

A. flaveolus Coq. was placed by Dyar (1928) as a light-yellow aberration of *titillans*. A ♀ with complete scaling may, perhaps, be referable to this form

but differs from Howard, Dyar and Knab's description of the type ♂ (1915) mainly as follows :—

Mesonotal scales partly dark bronzy-brown ; bare areas dark, somewhat differently arranged and, together with the dark-scaled areas, forming the background of a fairly distinct pattern. The pale mesonotal scales form large subquadrangular areas on the shoulders (fossae) and smaller patches and stripes elsewhere, while the bronzy scales occupy part of a broad median dark band on the anterior half, and form two narrow stripes at each side posteriorly, the scales here being to some extent intermixed with pale ones.

Abdomen clothed above with deep orange-coloured scales with slight coppery reflections ; first three segments partly denuded, but dark scales evidently absent ; tergites II–VII with very small basal, lateral, triangular patches of white scales. *Legs* with the dark scales practically black, contrasting strongly with the ochraceous yellow ones, which are intermixed with them in some regions, but may form ochraceous bands or areas. *Wings* with the broader scales somewhat less broad and emarginated than in *titillans*. A striking feature is the relatively very long *palpi*, which are quite half the length of the proboscis

Until ♂♂ with exactly similar thoracic markings can be studied, it seems best to regard the present form as a flavescent aberration of *titillans*, closely resembling *flaveolus*.

Two rather damaged ♀♀ from the Amazon valley, one taken by Professor R. M. Gordon at Manaos, 1921, the other on board ship by the late Dr. A. Aiken Clarke, agree in most respects with the specimens described above, as far as their characters can be seen. In one of the specimens the mesonotal scaling is incomplete, but groups of dark scales are present ; the mesonotum of the other ♀ is quite denuded.

Culex (Aëdinus) accelerans Root

A ♂ taken in the Utinga forest agreed exactly in the details of the ♂ terminalia with the description of the type material. A typical ♂ of *C.* (*Aëdinus*) *amazonensis* was also collected.

Sabethes tarsopus D. and K.

A single ♀ agreeing most closely with this form was taken in the Utinga forest, together with *S. cyaneus*.

In the specimen the bluish-white scales at the base of the tibia do not involve the tuft, and, from Dyar's (1928) remarks, it appears that this may represent a difference from *tarsopus* as it occurs in Venezuela. Further, the second segment of the front tarsus bears some bluish-white scales, which form an apical spot externally and a narrow line running almost the length of its vent al surface. In this character, the specimen shows an approach to the condition in *amazonicus*. Its occurrence lends support to Dyar's suggestion that the latter form is probably a variety (geographical race) of *tarsopus*.

Sabethes cyaneus Fab.

Six ♀♀ referable to this species were taken in the Utinga forest (1 in September, 1933, and 5 in September and November, 1934), and the following are the observations of one of us (G.R.W.) upon their habits.

Habits of adults. The ♀♀ were taken in a clump of heliconias in a fairly open part of the forest-path. The mosquitoes were very shy and were only obtained by waiting for about ten minutes among the heliconias. This species has a slow sailing flight and a habit of hovering perfectly motionless in the air for several minutes at a time, reminding one very much of humming-birds and dragon-flies. Goeldi (1905) records a similar flight in *S. goeldii* which he observed at Utinga. It seems highly probable that the paddles on the tarsi make it possible for these insects to hover in this way. No ♂♂ were observed. This species and also *S. tarsopus* bit the observer, but a slight movement sufficed to scare them away. The heliconias were searched for larvae, but none were found.

The species of *Sabethes* are apparently very rare and localized, as the forest around was searched on many occasions in apparently suitable places, but no specimens were found anywhere but in this particular spot.

Goeldia sp. (? **G. trichopus** Dyar)

The terminalia of a badly damaged ♂ referable to this genus were markedly different from existing descriptions and illustrations of the hypopygia of the species of *Goeldia*. It was therefore thought probable that our specimen represented a new species. In view of Komp's (1935) observations on Dyar's description of these structures in certain *Culex*, one of us (A.M.E.) wrote to Mr. Komp, who kindly advised us to consult Dr. Stone, to whom we are indebted for valuable information on the subject. From this we understand that considerable confusion has existed regarding the identity of *G. trichopus* Dyar and *G. longipes* Fab., and that the terminalia figured by Bonne and Bonne-Wepster (1925) as those of *trichopus* are actually those of *G. longipes*. Dr. Stone also states that the hypopygium of a specimen taken by Townsend, which he thinks is the true male of *G. trichopus*, agrees with a drawing sent to him of the structure in the present form in all characters including those which appeared to be distinctive in the Utinga male. In view of the fact that Townsend's (1934) description does not emphasize these points, we give the following notes on our specimen :—

ADULT. ♂. Colouration characters, as far as present, agreeing with those of *G. trichopus*, except that yellow scaling of abdominal tergites not markedly undulating. This point was noted by Dr. Edwards who kindly examined the specimen.

Terminalia (fig. 1). Coxites (side-pieces) rather less than three times as long as broad ; tergal surface with conspicuous patches of large bristles, with salient calices as shown in fig. 1, *c*. Before macerating the specimen the bristle-patches appeared as long, dense tufts and, from the few bristles *in situ*, they

evidently extended more than half-way to the tip of the coxite. Claspers expanded distally when seen in flattened aspect. Basal lobes sharply demarcated from coxite ; appearing triangular in flattened aspect (fig. 1, B) but somewhat club-shaped when viewed edgewise (A, left side) ; apex with about 12 long

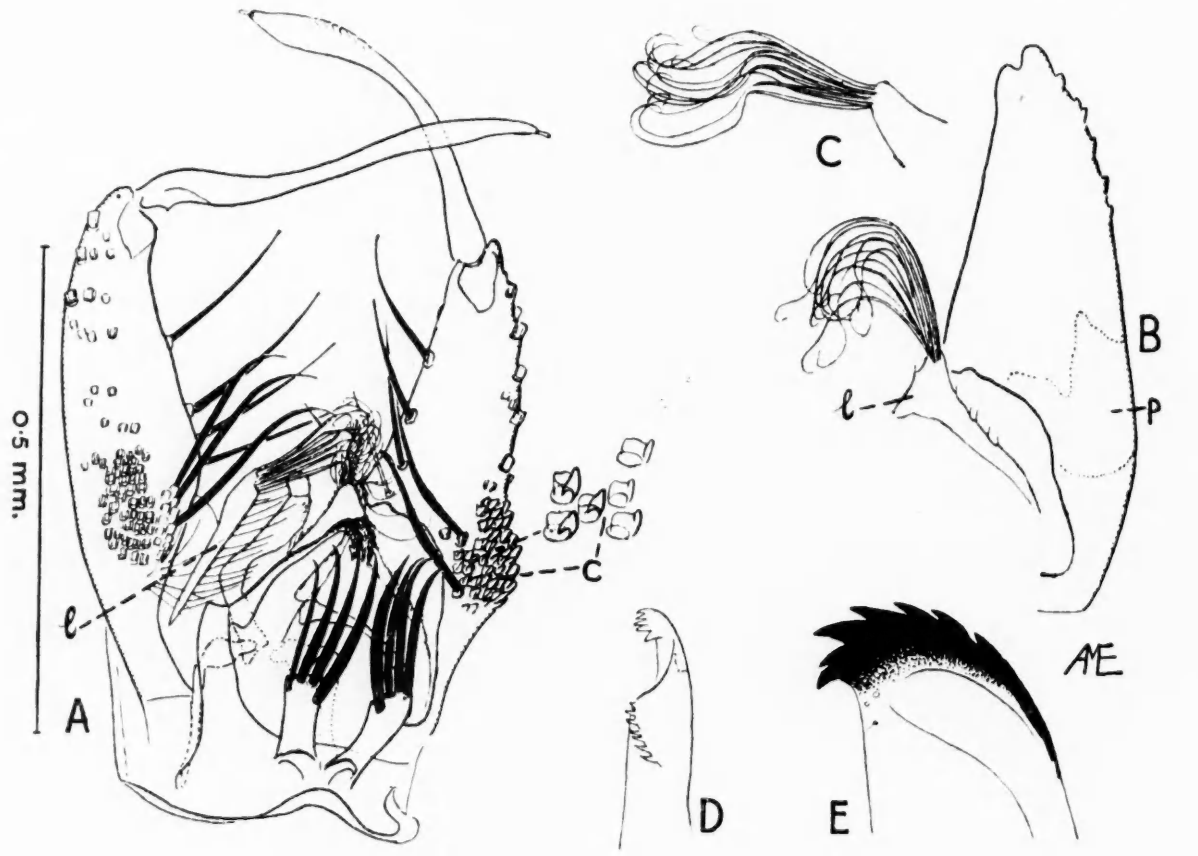

FIG. 1. *Goeldia* sp. (? *trichopus*). ♂ terminalia : A.—Whole terminalia, tergal aspect, slightly oblique ; one half of phallosome omitted ; B.—Outline of coxite with basal lobe ; C.—Apex of basal lobe showing hairs in flattened aspect ; D.—Apex of half of phallosome ; E.—Apex of tenth sternite. (A.–C. to same scale ; D. and E. to larger scale.) *c*.—calices of large bristles ; *l*.—basal lobe of coxite ; *p*.—position of bristle-patch.

finely attenuated hairs, characteristically bent (fig. 1, C) and appearing as a tangled mass of threads when viewed from certain aspects. Phallosome with two sets of small serrations (fig. 1, D). Ninth tergites with 4 spines. Tenth sternites (cerci or paraprocts) heavily serrated distally, as shown in fig. 1, E.

Habits. About half a dozen specimens, apparently all ♂♂, were observed flying backwards and forwards about 3 feet from the ground, in the same clump of heliconias in the path through the Utinga forest near Pará. In September, 1934, 2 ♂♂ were taken at this spot, and two months later specimens were again seen behaving in the same way.

Aëdes (Ochlerotatus) albifasciatus Macq.

Recently, when on a voyage down the east coast of America, one of us (G.R.W.) observed that while the ship was in harbour at Rio Grande do Sul, Brazil, numerous ♀♀ of this large *Aëdes* came on board. The mosquitoes were troublesome biters, and it was generally, but, no doubt, erroneously, imagined that they had emerged from the coal-bunkers. It should be noted that Rio Grande do Sul was the most southerly port visited during the voyage.

Dr. Edwards kindly compared specimens with *A. albifasciatus* taken by himself in Chile, and found that the Brazilian material agreed with some examples of the paler form of this species.

All the specimens from Rio Grande do Sul were of the light type, having the femora, tibiae and first tarsals conspicuously speckled with pale scales. The wing veins show many pale scales, the stems of the second, fourth and fifth veins being predominantly pale in all but one of the examples. The dark scales of the median area of the mesonotum were very dark blackish-brown in all cases where present.

Shannon (1931) does not include *A. albifasciatus* in the list of Brazilian *Aëdes*, and Edwards (1930) points out that doubt exists as to the occurrence of the species in Brazil, in which country it ' has not been found by any recent workers.' The present record shows that the pale form of the species occurs in numbers in the Rio Grande do Sul, and it would be of great interest to know whether it is widely distributed in this part of the country or whether it has been introduced recently, as in the case of *A. costalis* (*gambiae*) in Natal, Brazil.

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NOTES ON ANOPHELINES

I.—DESCRIPTION OF *ANOPHELES MARSHALLI* VAR. *GIBBINSI* FROM UGANDA

II.—THE CHARACTERS OF *A. MACULIPENNIS* VAR. *MESSEAE* IN WIRRAL, ENGLAND

BY

A. M. EVANS

(*Received for publication* 22 *October*, 1935)

I. DESCRIPTION OF *ANOPHELES MARSHALLI* VAR. *GIBBINSI* FROM UGANDA

Dr. De Meillon has recently informed me of the results of his studies on the type form of *A. marshalli* as it occurs in the Transvaal, and of var. *pitchfordi* from the neighbourhood of Eshowe, Natal. From this information it is clear that the form of *marshalli* studied by Mr. Gibbins in Uganda (1932, 1933*a*) is sufficiently distinct in the characters of the larva and pupa to merit varietal status.

Anopheles marshalli var. **gibbinsi** var. nov.

This variety differs from the type form mainly in the character of the inner clypeal hairs, which are short and stumpy.

ADULT. ♀. Very similar to type form.

Thorax. Mesonotal scales, except on anterior promontory, mainly very narrow, almost hair-like, with slight yellowish tinge ; scales at sides on anterior half less narrow ; a patch of long narrow scales above and in front of wing root.

Legs. Apical pale rings on first 4 hind tarsal segments sometimes very narrow.

Wings. Costa with pale spots on outer half small in all the specimens ; not more than one pale basal costal interruption, which may be greatly reduced. Fringe spots at apex of sixth vein sometimes absent, and in one case also that at lower branch of fifth.

ADULT. ♂. *Terminalia.* Differing from that of Southern Rhodesia specimens examined as follows :—*harpagones* with at least one inner accessory hair ; *phallosome* with the number of leaflets at each side varying from 5 to at least 8. In one apparently anomalous harpago a long supernumary external hair was present.

PUPA. Differing from that of the type form (Transvaal) mainly as follows :—

Paddle. Apical bristle short and practically straight (only 1/7 length of paddle).

Bristle C. V-VII definitely shorter than segments, the ratios varying from about 2/3–6/7, occasionally bifid.

LARVA. *Head* (fig. 1, A). *Clypeal hairs*, *inner* relatively very short and rather abruptly pointed, appearing stumpy ; length only about 1/4 of that of the fronto-clypeus and not more than 0·17 mm. *Outer* also stout and rather abruptly pointed ; about 2/3 the length of inner. *Posterior* short and delicate. *Antenna* with spicules stout and prominent on inner side and longest on basal half, but with no very definite grouping of long spines.

Thorax. Palmate hair practically equal in size to that of the type form (Transvaal).

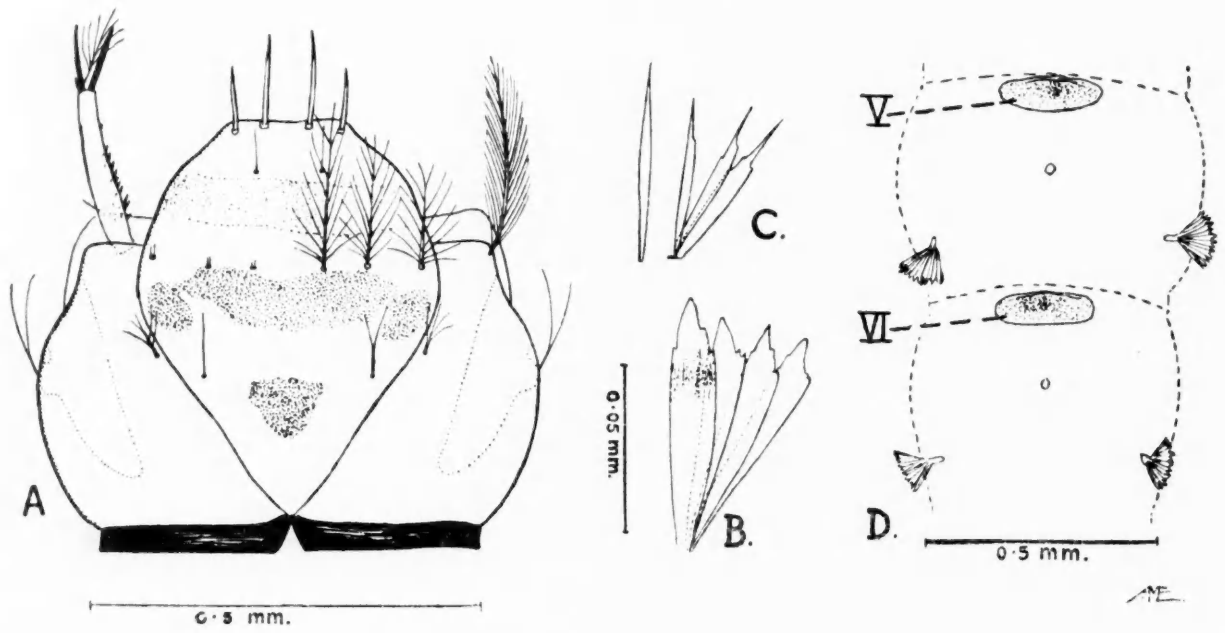

FIG. 1. *Anopheles marshalli* var. *gibbinsi* var. nov., larval details. A.—Head, drawn from somewhat flattened mount ; some details omitted ; B.—Leaflets from palmate hair of fourth abdominal segment ; C.—Leaflets from segment I (2 specimens) ; D.—Outline of segments V and VI to show width of tergal plates in relation to distance between palmate hairs.

Abdomen (fig. 1, B–D). *Palmate hairs*. I, with about 10 narrow leaflets which may have 1–3 serrations and a definite filament, or may be entirely unshouldered. IV and V, small, leaflets about 0·7 mm. with broad blade and very short filaments, which are broad at the base and commonly blunt. *Tergal plates* of moderate width ; on V the width of the plate is slightly less than half the distance between the bases of the palmate hairs. *Saddle hair* branched as in type form. *Pecten* very similar to that of South African *marshalli* ; short teeth about 2/5 to half the length of the ventral long tooth.

EGG. Gibbins (1933*b*) has described and illustrated the egg of this form. From De Meillon's (1934) description of South African material, the egg of the variety is evidently of the same type but differs mainly in the very variable size and character of the frills and in the condition of the enclosed areas. In some cases the areas bounded by the frills are completely isolated but smaller than

in the type form ; in other cases these areas are continuous through a 'bottle-neck ' with the main part of the enclosed (upper) surface.

HABITS and RELATION TO MALARIA. Gibbins (1932, 1933*a*) has shown that this variety is a common house-frequenter and an important vector of malaria at Fort Portal.

UGANDA. Fort Portal, 2 ♂♂ and 2 ♀♀ (including type and paratypes) with larval and pupal pelts, October 9th, 1931, E. G. Gibbins. Larvae from Kabale, 1932, and one from Hoima, 1932, G. H. E. Hopkins, are evidently referable to this form.

Type ♀ in Liverpool School of Tropical Medicine ; paratype ♀ in British Museum. The variety is named in honour of its discoverer, Mr. E. G. Gibbins.

II. THE CHARACTERS OF *ANOPHELES MACULIPENNIS* VAR. *MESSEAE* IN WIRRAL, ENGLAND

In a previous paper (1934) I recorded the presence in Wirral, Cheshire, of a form of *A. maculipennis* which agreed in the character of the egg and in hibernation habits with var. *messeae* as described by Missiroli, Hackett and Martini (1933) and by de Buck, Schoute and Swellengrebel (1932). Hackett and Missiroli (1935) give several records of the variety from eastern and southern counties.

It appears from the recent definitions of the characters of var. *messeae* (de Buck *et al.*, 1934 ; Hackett, 1934) that the variety may exhibit geographical differentiation. I have, therefore, examined further material of the Wirral form, including larvae and ♂♂ reared from eggs, and find that, in most of the diagnostic characters, our *messeae* agree closely with the Dutch form as defined by de Buck, Schoute and Swellengrebel (1934). The number of specimens examined was small, but, as a great range of variation was exhibited, the percentages are given for most of the characters, although they probably give only an approximate indication for the form as a whole.

♂. TERMINALIA. The external spine was examined in 39 cases and could be roughly classed into five intergrading groups, as follows : acuminate, 8 ; pointed but not acuminate, 5 ; bluntly pointed, 10 ; blunt but not rounded, 4 ; round-tipped, 12.

Thus, only 30·8 per cent. were definitely round-tipped, the percentage being near to that of Dutch *messeae* (37 per cent.) as defined by de Buck *et al.* (1934). In 57·8 per cent. of cases the external spine was single. In a number of cases where the spine was double there was a tendency to form a rudimentary club, and in 3 cases a club was actually present.

The preparations, in almost all cases, were made from dried material macerated in K.O.H. and mounted either in De Faure's fluid or, after staining with carbol fuchsin, in canada balsam.

As Martini (1933) raises the question whether apparent variation in the

character of the spine may be partly due to the effects of orientation, it should be mentioned that, in determining the character of the spines of *atroparvus* (Evans, 1934) and of the present form, particular attention has been given to this point. It is, of course, obvious that the most characteristic shape of the spine is exhibited when the isolated harpago is mounted flat, without pressure.

LARVAE. *Palmate hair of abdominal segment II.* The character has been determined in 51 cases, and in 31 of these the hair was not appreciably modified ; the condition of the hair is evidently very close to that in Dutch *messeae*.

Antepalmate hairs. Fifty-eight cases were examined, and the average number of branches of the hairs of one side on segments IV and V combined was 9·2. This number is in exact agreement with that given by de Buck *et al.* (1934) for Dutch *messeae*.

EGGS. Altogether 11 batches of eggs were laid by 10 ♀♀. The eggs appeared to agree with those of Dutch *messeae*, but, in order to be quite sure on this point, samples from a typical batch were sent to Amsterdam. Professor Swellengrœbel kindly informed me that both he and Dr. de Buck had examined the eggs and found that ' they are quite the same as ours ' ; but he states that this does not prove that the *messeae* from Wirral and from Holland are identical.

BEHAVIOUR OF HIBERNATING ♀♀. The hibernating habits of this form in nature are of the complete type (Evans, 1934). By February 14th, 1934, the ♀♀ had evidently reached the end of their hibernation, as they then took blood-meals in the laboratory at 64–68° F. and in the majority of cases oviposited in from $5\frac{1}{2}$ to 11 days after one blood-meal (at 60–66° F.). At 76–78° F. oviposition followed in $3\frac{1}{2}$ to 5 days after a single meal of blood (human or rabbit).

A number of ♀♀ collected from the same locality in October, 1934, were brought to the laboratory, and, at a temperature of 67·5° F., 4 fed at once on a human arm (enforced feeding of de Buck *et al.*, 1934). The state of the blood in the stomach was noted at intervals after feeding, and it was seen that in 3 of the ♀♀ the anomalous condition noted by these authors in hibernating Dutch *messeae* was maintained for from 3 to 5 days. In the fourth specimen the digestion appeared to be of a normal character, but this specimen and the others died in from 7 to 21 days without ovipositing. In December, 1934, two other ♀♀ of this lot oviposited after taking two meals of blood.

CONCLUSION

The form of *A. maculipennis* studied in Wirral, Cheshire, is evidently morphologically almost identical with the Dutch form of var. *messeae*. The behaviour during hibernation is of the same general type as in Dutch *messeae*, although there appears to be greater variation in reaction to experimental conditions, and the period of complete hibernation may terminate on a somewhat earlier date. The variable reaction to experimental conditions supports the suggestion made by Professor Swellengrebel (*in litt.*, December 22nd, 1934)

that the occurrence of normal digestion and precocious ovulation as early as December may indicate a biological difference between the forms of *messeae* occurring in Holland and Wirral, England.

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TRYPANOSOMIASIS OF STOCK IN MAURITIUS*

II.—OBSERVATIONS ON THE INCIDENCE AND DISTRIBUTION OF TRYPANOSOMIASIS IN CATTLE

BY

A. R. D. ADAMS, M.D.

(*Received for publication* 29 *October*, 1935)

In a previous communication (Adams, 1935) attention has been drawn to the fact that in addition to classical surra, which has been present in Mauritius for over thirty years, a second trypanosomal disease of stock occurs in the colony. This condition, a form of nagana due to *T. vivax*, has doubtless been imported from Africa in consignments of cattle received from that continent. Since the first recognition of this second trypanosome in the island, particular attention has been paid to the specific diagnosis of all trypanosomes recovered from domestic animals, and the present paper records the findings over a twelve-month period in respect of the incidence and distribution of these parasites in cattle.

INCIDENCE

Arrangements made with the local Department of Agriculture resulted in the submission of slides, examined by them and found to contain trypanosomes, for fuller detailed investigation at this laboratory. From July 31st, 1934, until May 1st, 1935, the members of the veterinary section of that department examined thin blood-films from 5,471 local cattle selected at random from the indigenous bovine population of the colony. Of these 5,471 animals, 65 were found by the veterinary inspectors to harbour trypanosomes, and slides from these beasts were sent for further examination at this laboratory. *T. vivax* was recognized in 40 of the affected animals, and *T. evansi* in the remaining 25. This gives an incidence of 0·72 per cent. for *T. vivax* and 0·46 per cent. for *T. evansi* in all cattle examined.

The above arrangement proving unsatisfactory for sundry reasons, it was decided that from May 1st, 1935, until the end of the ensuing July *all* specimens taken by the veterinary authority, irrespective of previous examination by the veterinarians, should be submitted to the laboratory. This scheme relieved the veterinary staff of the necessity of examining the material, and thus afforded them more time for field-work and the collection of material. The laboratory also benefited to the extent that the fixation and staining of slides could be performed in a manner adjudged more appropriate to morphological study and

* From the Bacteriological Laboratory, Medical and Health Department, Mauritius.

exact determination of species. During this three-month period single thin blood-films from 2,170 cattle were stained and examined by us ; 44 (2·03 per cent.) were determined to contain *T. vivax*, while 16 (0·74 per cent.) contained *T. evansi*. These figures for incidence are considerably greater than those obtained during the previous nine-month period of the investigation, in spite of the fact that the examinations were made in the cool weather, when oxen were not working extensively in the fields, and when the local flies and insects were at their lowest numerical strength.

During this same period (May–July, 1935) several cattle were discovered infected with *T. theileri*, a species of trypanosome hitherto unrecorded in Mauritius. A number of beasts were found infected with filariae of various species ; *Babesia bigemina* and *B. mutans* were occasionally seen ; and on a single occasion many Rainey's corpuscles (*Sarcocystis meischeriana*) were observed in a thin blood-film from the ear of an ox. This latter observation is of academic interest, and Sergent (1921) has recorded a similar finding.

Throughout the work only a single thin-film preparation of peripheral blood, taken from the ear, was examined. The total time necessary thoroughly to examine these slides in the usual manner—employing a 2 mm. objective—was found to be greater than could be afforded, during the second phase of the investigation. It was determined that at least 10 minutes were required for a reasonably comprehensive study of each film, using this oil-immersion lens, and that the fatigue resulting from search of some two or three dozen consecutive specimens greatly militated against that accuracy desirable in the work. After some trials a method of procedure was adopted which was found to yield satisfactory results with a minimum expenditure of time, energy and material. This was as follows :—

Each slide on receipt was numbered. The films were fixed, in batches, for 10 minutes in absolute alcohol ; and were subsequently stained for at least half an hour in Giemsa. Paraffinum liquidum (B.P.) was thinly smeared on the surface of each, and examination was made with the 4 mm. dry objective. Five minutes were devoted to each slide, and properly stained trypanosomes were readily recognizable by this magnification. The species could frequently be judged with this lens, but the determination was confirmed by the use of the 2 mm. oil-immersion objective used in the paraffin medium, although naturally the resolution obtained was not that to be expected in the correct immersion fluids. On occasions, where more exact resolution was deemed advisable, the proper immersion fluid was employed after removal of the paraffin by a suitable solvent. Paraffin was employed in the interests of economy, but, where this has not to be considered to the same extent, cedar-wood oil is preferable.

In the Table are recorded in tabular form the findings for *T. vivax* and *T. evansi* throughout the twelve-month period of the survey. No other trypanosomes were found, with the exception of *T. theileri* mentioned above. There is thus no indication of the presence in the colony of any other pathogenic trypanosome.

TABLE

To show the number of cattle examined,* and the incidence of the two pathogenic trypanosomes found in them, during the periods July 31st, 1934–May 1st, 1935, and May 1st–July 31st, 1935

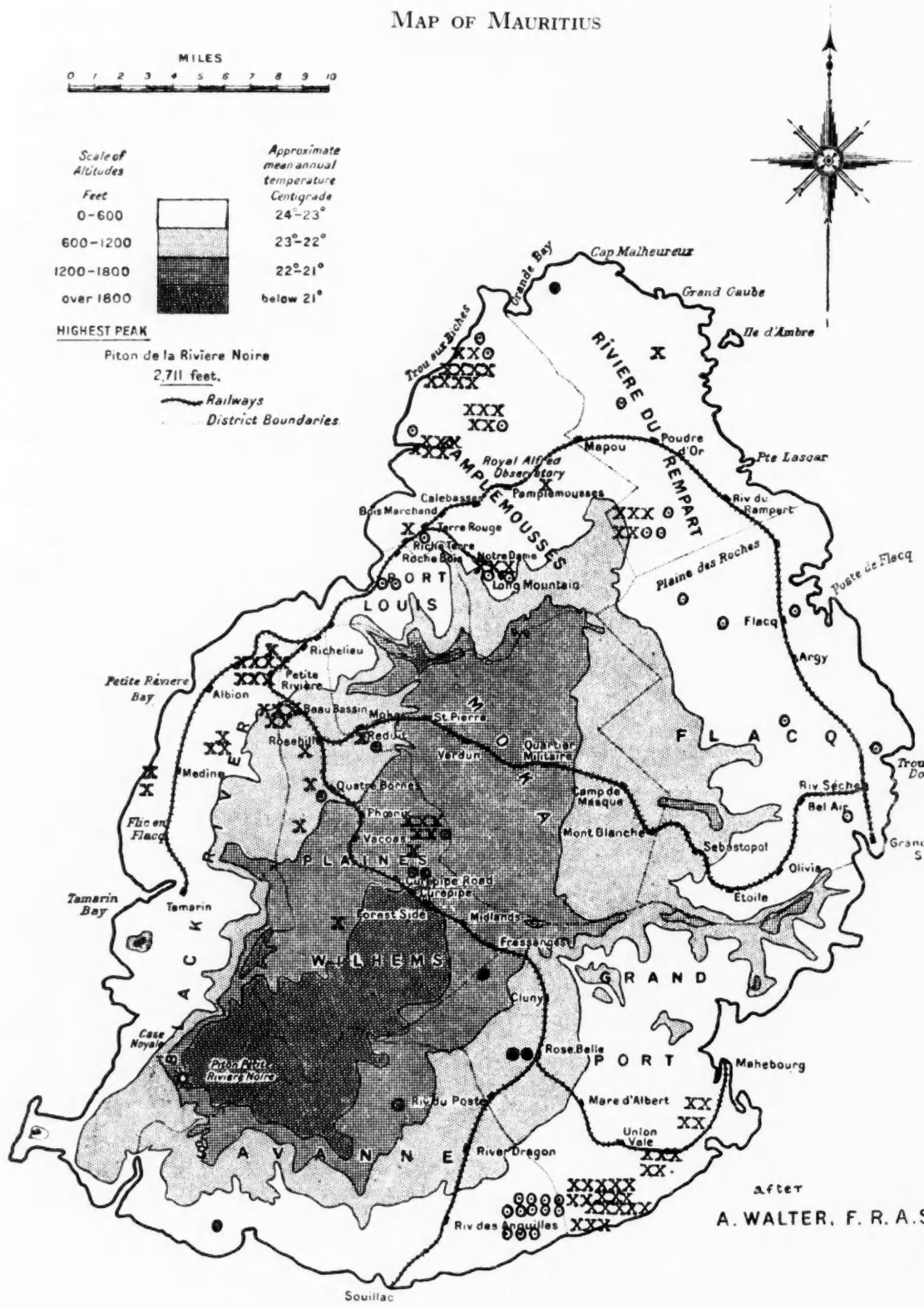
Period	Total no. of cattle examined	No. infected with *T. vivax*	No. infected with *T. evansi*
July 31st, 1934, to May 1st, 1935	5,471	40 (0·72 per cent.	25 (0·46 per cent.)
May 1st, 1935, to July 31st, 1935	2,170	44 (2·03 per cent.)	16 (0·74 per cent.)

* All figures are based on one examination of the peripheral blood, as a thin-film preparation, of each animal.

DISTRIBUTION

On the map are shown the places in which cattle and equines infected with *T. vivax* and *T. evansi* were found between July 31st, 1934, and July 31st, 1935. At first sight it would appear that, while *T. evansi* has been found relatively evenly distributed throughout the island, *T. vivax* is absent from the eastern side and the south-western portion of the colony. This apparent difference in the distributions of the two parasites is explicable on two grounds. The main road with good surface runs from the north end of the island, *via* the capital, Port Louis, to the south ; it very roughly follows the main railway-line in its course, and along it are situated the main residential districts. The veterinary inspectors travelled on duty by rail, motor-'bus or cycle ; and on inquiry it was found that, as was to be expected, the areas covered by them were chiefly in close proximity to the available means of rapid transport. The more inaccessible estates and villages were only briefly and superficially touched upon on the rare occasions when they were visited at all. As *T. vivax* infection in cattle did not cause the severe and sudden disability in affected animals which attracted the attention of the layman, owners of affected animals did not pay the attention to it that the marked and crippling debility occasioned by *T. evansi* imperatively demanded. Surra is well known and usually quickly diagnosed by small-owners of draft animals ; in the interests of their livelihood these men are only too ready to demand veterinary aid in restoring their beasts to a condition which enables them to show a financial profit for their upkeep. It thus follows that isolated cases of *T. evansi* infection were reported from all parts of the country, and that these were visited by members of the veterinary staff to confirm the diagnosis, enforce quarantine regulations and administer treatment. Cases of *T. vivax* infestation, on the other hand, were not notified to authority by the

MAP OF MAURITIUS



To show the distribution of cases of trypanosomiasis detected in stock between July, 1934 and July, 1935. × = *T. vivax* in cattle ; ○ = *T. evansi* in cattle ; ● = *T. evansi* in equines

owners, in view of the much milder manifestations of the disease, and were detected only by systematic examination of numbers of animals in selected districts.

The south-western area of the island, embracing Black River and the adjoining portions of Savanne districts, is made up of considerably rougher and drier country than the remainder of the island. Sugar-cane is grown to lesser extent, and consequently the number of draft cattle is proportionately less than elsewhere.

Four cases of *T. evansi* infection in equines are shown. All these cases with one exception—a mule—were in horses or ponies. They were all reported voluntarily to the veterinary authorities as sick animals ; and equines in general have not been examined in a systematic manner. No case of *T. vivax* infestation has so far been found in a local equine.

DISCUSSION

From the facts recorded above it is abundantly evident that both *T. vivax* and *T. evansi* are widespread throughout the colony of Mauritius. It is further patent that *T. vivax* is present in a very considerably greater number of cattle than is *T. evansi*. When it is recalled that a *T. evansi* infection in the majority of cases is probably recognizable by thorough examination of a single specimen of blood, but that in the case of *T. vivax* repeated examinations of the peripheral blood may fail to reveal a current infection, the preponderance of *T. vivax* infestation over that of *T. evansi* may justifiably be assumed to be very much greater than is apparent from a study of the above figures. In support of this contention it may be stated that experiments at this laboratory have shown that with local strains of *T. vivax* the parasite may disappear from the peripheral blood of infected domestic stock for weeks or months, to recur for two or three days and to disappear again for a similar period. During its absence, as judged by microscopical examination, subinoculation of large amounts of blood into susceptible animals has on several occasions failed to produce infection, a condition of affairs which has not been found to obtain with *T. evansi* infections.

The figure for *T. vivax* incidence may therefore be assumed to be very considerably below the true figure of incidence in cattle, while that for *T. evansi* may, for practical purposes, be taken as approaching the truth. No figures are quoted for the incidence of *T. theileri* in view of the notorious difficulty in determining the absence or presence of this trypanosome by microscopical examination alone of the peripheral blood. There is little doubt that it is as common in this island as it has been found elsewhere throughout the world.

On considering the incidence and distribution of *T. vivax* in cattle in the colony, any supposition that this trypanosome is of recent introduction into Mauritius becomes, in the writer's opinion, untenable. Available records of trypanosomiasis of stock in the island throw no helpful light on the determination of the period of its first appearance. Study of published data in the reports of

various Government departments merely confirms the belief that *T. vivax* may well have existed in the country for many years entirely unnoticed. Until the last year or so the examinations of the bloods of various forms of domestic stock were made solely with the object of determining whether obvious trypanosomal infestation existed, and no provision whatsoever was made for specific identification of the parasites found. There can be little doubt that the effects of the routine treatment administered to animals found suffering from trypanosomiasis were not properly assessed with the aid of critical microscopical examinations at suitable intervals ; and the impression is gained from a study of what data are available that all trypanosomes, *T. evansi*, *T. vivax* and *T. theileri* when seen, have been dubbed *T. evansi* without critical study.

Even when classical surra first invaded the island in epizootic form in 1901, *T. vivax* may well have been in the colony, and may have been overlooked in the general holocaust due to the newly arrived plague. Edington (1902), and later in his paper with Coutts (1907), repeatedly refers to his own belief that a trypanosomal disease was present in the stock of the island before the well-known surra epizootic of 1901. On study of Edington's papers and reports it is evident that that author's knowledge of the subject was not such as would be expected of a worker some 30 years later ; his observations on the possible modes of infection, treatment and kindred subjects bear witness to this fact ; nevertheless Edington's inquiries made on the spot, and with the facilities accorded him in their conduct, render his opinion of some weight. His publications, moreover, constitute the only available published record of scientific inquiry into the trypanosomiasis of the island during the first quarter of the century of its recognition, with the exception of sundry records of experiments on the treatment of ' surra ' published in departmental reports. It would be exceedingly difficult to disprove an assertion that *T. vivax* has been enzootic in Mauritius for over 30 years ; and the present position of this trypanosome in the island lends support to the deduction that it has been established here for a very considerable period.

I am greatly indebted to the veterinary section of the local Department of Agriculture for the provision of the material on which this paper is based. My scientific assistant, Mr. Webb, has been of the greatest assistance in the course of the work, and I am grateful to him for his willing collaboration.

SUMMARY

1. A survey of the incidence and distribution of trypanosomal diseases of cattle in Mauritius was undertaken in collaboration with the Agricultural Department of the colony.

2. Single thin blood-films were taken from the ears of local cattle selected at random from the indigenous bovine population ; these were stained and examined in a manner described.

3. During the period May 1st, 1935–July 31st, 1935, 2,170 cattle were examined in this manner. Of these animals 2·03 per cent. were found to be infected with *T. vivax*, and 0·74 per cent. with *T. evansi*.

4. *T. theileri* was also recovered from several beasts, thus conclusively demonstrating its presence in the colony.

5. During the survey several species of filariae were observed; *Babesia mutans* and *B. bigemina* were also seen; and in a single case *Sarcocystis meischeriana* was found in a film.

6. Attention is drawn to the fact that the figure obtained for the incidence of *T. vivax* must be very much inferior to the true figure for this trypanosome, as *T. vivax* retires from the peripheral blood of infected stock for weeks or months, to reappear for a few days, and again to disappear for prolonged periods.

7. A map is given showing the distribution of *T. vivax* and *T. evansi* in the island over a period of one year. Some explanation is afforded of the apparent restriction of the former parasite to the more populous districts of the country.

8. From a study of available data, and of the incidence and distribution of *T. vivax* at the present day, the conclusion is reached that this trypanosome may have been present in the colony for many years. It is possible that it was present before surra was introduced in 1901. It is almost certainly not of very recent introduction.

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STUDIES ON THE HIGHER DIPTERA OF MEDICAL AND VETERINARY IMPORTANCE

A REVISION OF THE SPECIES OF THE GENUS GLOSSINA WIEDEMANN BASED ON A COMPARATIVE STUDY OF THE MALE AND FEMALE TERMINALIA

(*Continued from page* 315)

BY

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INTRODUCTION

In this paper I propose to illustrate the ♂ and ♀ terminalia of *Dermatobia hominis* L. and of several species of *Cuterebra*. For the material on which the study is based I am indebted to Dr. Paulo de Toledo Artigas and Professor Flavio da Fonseca, Instituto Butantan, São Paulo, Brazil, who have most generously, and with considerable trouble, bred out and sent to me for study a long series of ♂♂ and ♀♀ of *Dermatobia hominis*. For the specimens of *Cuterebra* I am indebted to Mr. Cornelius B. Philip, Entomologist to the Rocky Mountain Fever Laboratory, Hamilton, Montana, who has sent me ♂♂ and ♀♀ of several species, which he bred out as well as caught. Although the larvae of species of *Cuterebra* are fairly commonly met with, the adults, on the other hand, are rare insects in collections. I therefore appreciate Mr. Philip's thoughtfulness and generosity in letting me have this material for the study of the terminalia ; most entomologists are loath to part with such specimens, especially when they know they are likely to be mutilated. I wish, therefore, to thank these gentlemen most cordially for giving me this opportunity of making a critical comparative study of the terminalia of these interesting parasitic Diptera ; the material has enabled me to settle some important points connected with the systematics of these flies. Many years ago Dr. Seymour Hadwen gave me a ♀ of *C. fontinella*, and the late Dr. Aldrich a ♂ of *C. americana*. I wish also to thank Major E. E. Austen for determining the specimens of *Cuterebra* for me.

It will be remembered that the warble flies of the New World are placed in the subfamily Cuterebrinae, and that all the species are, in their larval stages, dermal parasites of man and animals of all kinds, but especially of rodents. *Dermatobia hominis*, often called the tropical warble fly, is the only one whose larva parasitizes man. It is widely distributed in the tropical and subtropical areas of the New World, and its larva has most probably been known to man for centuries. Dunn (1934), who has recently recorded some new facts regarding this fly, states that : ' Any party of individuals that spends much time in the low, humid, coastal or forest regions of Panama is quite likely to have some members

infested with the larvae after a few days.' In his own case he was able to demonstrate that ' clothing does not furnish the protection against infestation that one would naturally suppose.' In the case of domestic animals, Dunn points out that the larvae of *D. hominis* cause very serious losses among cattle in Panama. It will be remembered that this fly lays its eggs on the abdomen of insects, especially of mosquitoes, as well as biting, haematophagous and sweat-feeding higher Diptera such as *Stomoxys*, *Anthomyia*, *Limnophora*, *Synthesiomyia*, etc. The first stage larva passes rapidly out of the egg-shell on to the skin (or clothing) of the host on which the carrier alights to feed. The life-history has been very fully worked out.

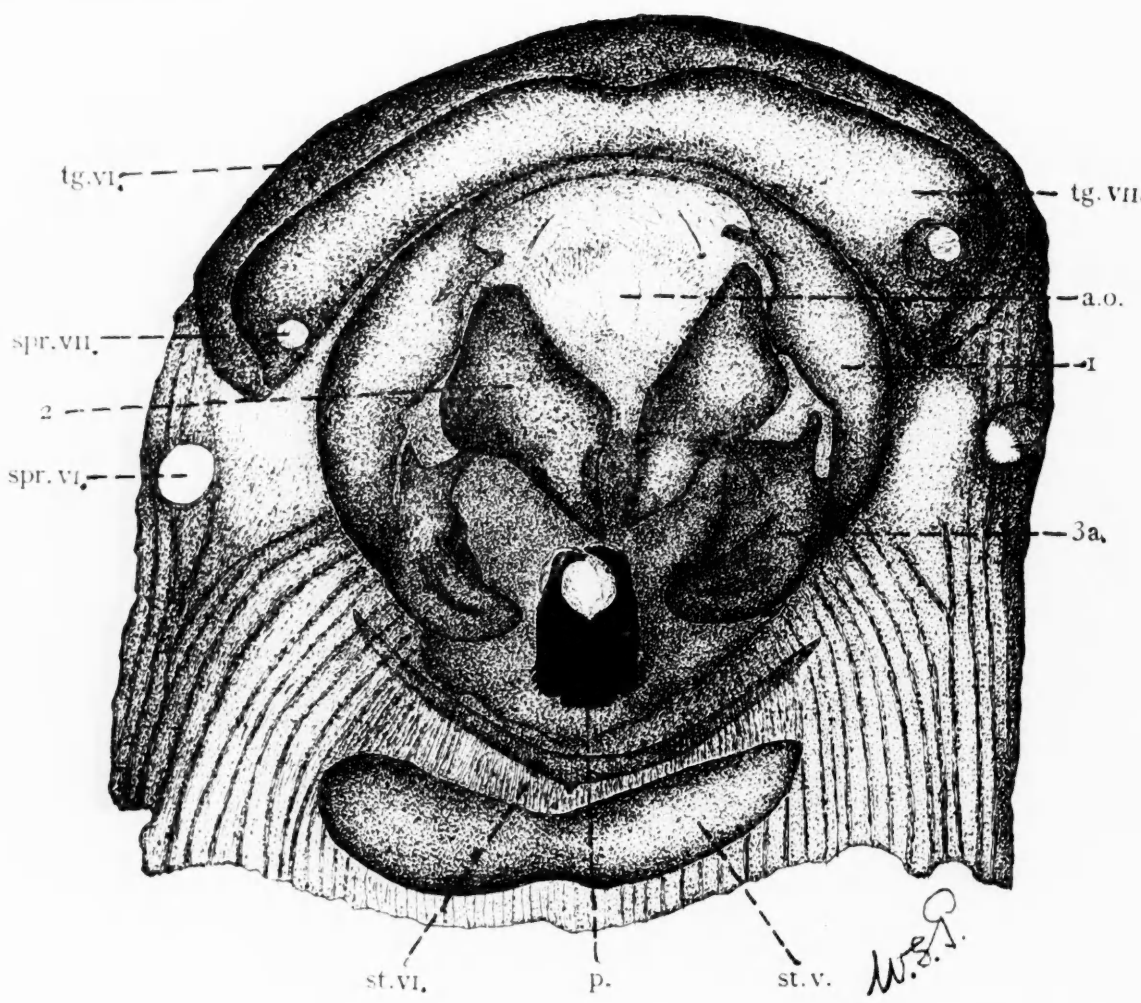

Fig. 1.—Ventral view of end of abdomen of ♂ *D. hominis* showing relationships of parts ; *a.o.*—Anal opening ; *p.*—End of phallosome ; *st. v*, *st. vi.*—Fifth and sixth sterna ; *spr. vi*, *spr. vii.*—Sixth, seventh spiracles ; *tg. vi*, *tg. vii.*—Sixth, seventh terga ; 1.—Tenth tergum ; 2.—Anal cercus ; 3*a.*—Distal segment of ninth coxite (São Paulo, Brazil).

Very little is yet known of the life-histories of the species of *Cuterebra*. Ferris (1920) has described the first stage larva of *C. americana* and noted that it hatches of its own accord from the egg. Parker and Wells (1919) carried out some experiments with the first stage larvae of *C. tenebrosa* and noted that,

when the larvae were fed by the mouth to prairie-dogs, a certain percentage eventually appeared under the skin and completed their development, suggesting that, in this species at least, infestation is *via* the alimentary canal, and that the larva does not hatch of its own accord from the egg. They did not carry out

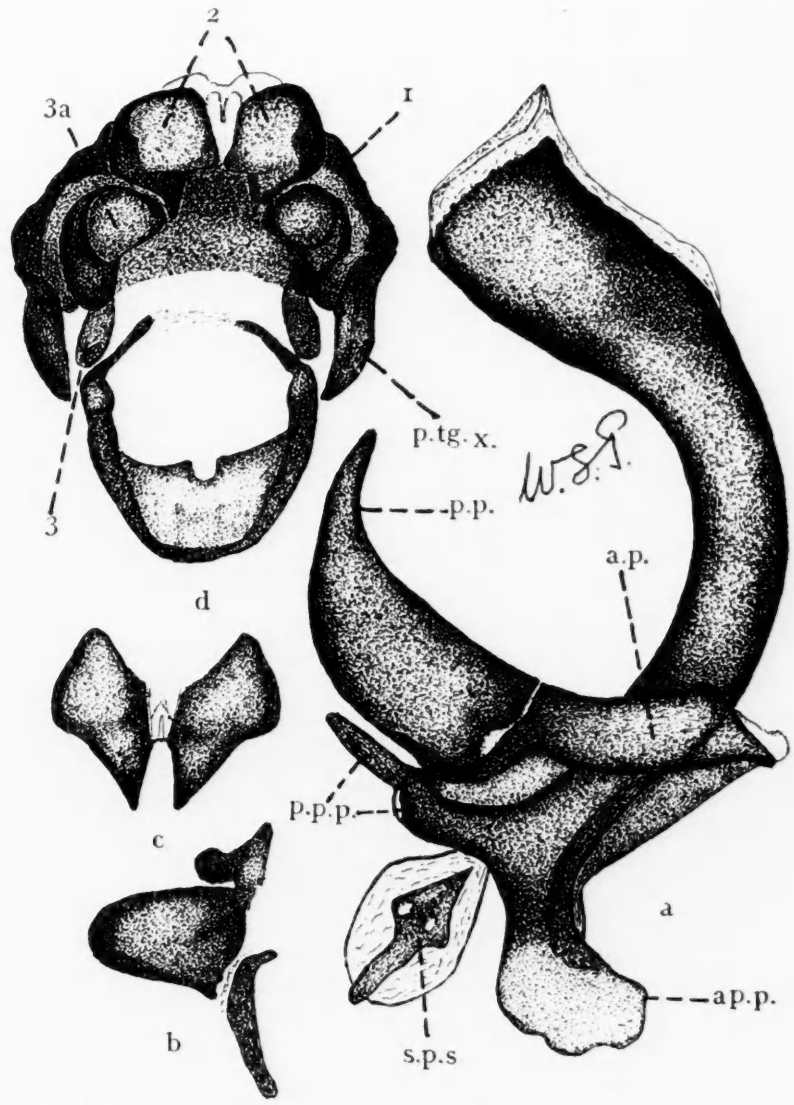

FIG. 2. *a.*—Phallosome and one paramere of *hominis* in side view; *a.p.*—Anterior part of paramere; *ap.p.*—Apodeme of phallosome; *p.p.*—Posterior part of paramere; *p.p.p.*—Posterior process of phallosome; *p.tg.x.*—Process of tenth tergum; *s.p.s.*—Sperm pump sclerite; *b.*—Two parts of ninth coxite; *c.*—Ventral view of anal cerci; *d.*—Showing relations of ninth tergo-sternum and tenth tergum; 1.—Tenth tergum; 2.—Anal cerci; 3.—Proximal segment of ninth coxite; 3*a.*—Distal segment of same; note process of tenth tergum articulating with side of tenth tergo-sternum (São Paulo, Brazil).

any experiments to see if the larva could enter directly into the skin when applied to it. These observations clearly suggest that, in the case of those species in which the first stage larva does not of its own accord leave the egg-shell, friction of some sort is necessary to free it before it enters the alimentary canal,

whereas, in the case of those species in which the first stage larva of its own accord leaves the egg, it possibly enters the skin directly. These and other points connected with the habits of these larvae will only be settled by further exact experiments. It is not known where the eggs are laid, but there are only two possibilities here—they are laid either directly on the hair of the host, or on some object in its habitat (the latter word referring to burrows of rodents, resting-places of other animals such as monkeys, etc.). I hope that a thorough investigation of the life-histories of these flies will soon be undertaken.

At present some 45 species of *Cuterebra* are known, and the genus has now been split into numerous subgenera, which some authors have raised to generic rank. The North-American species belong to the genera *Cuterebra* Clark (sens.

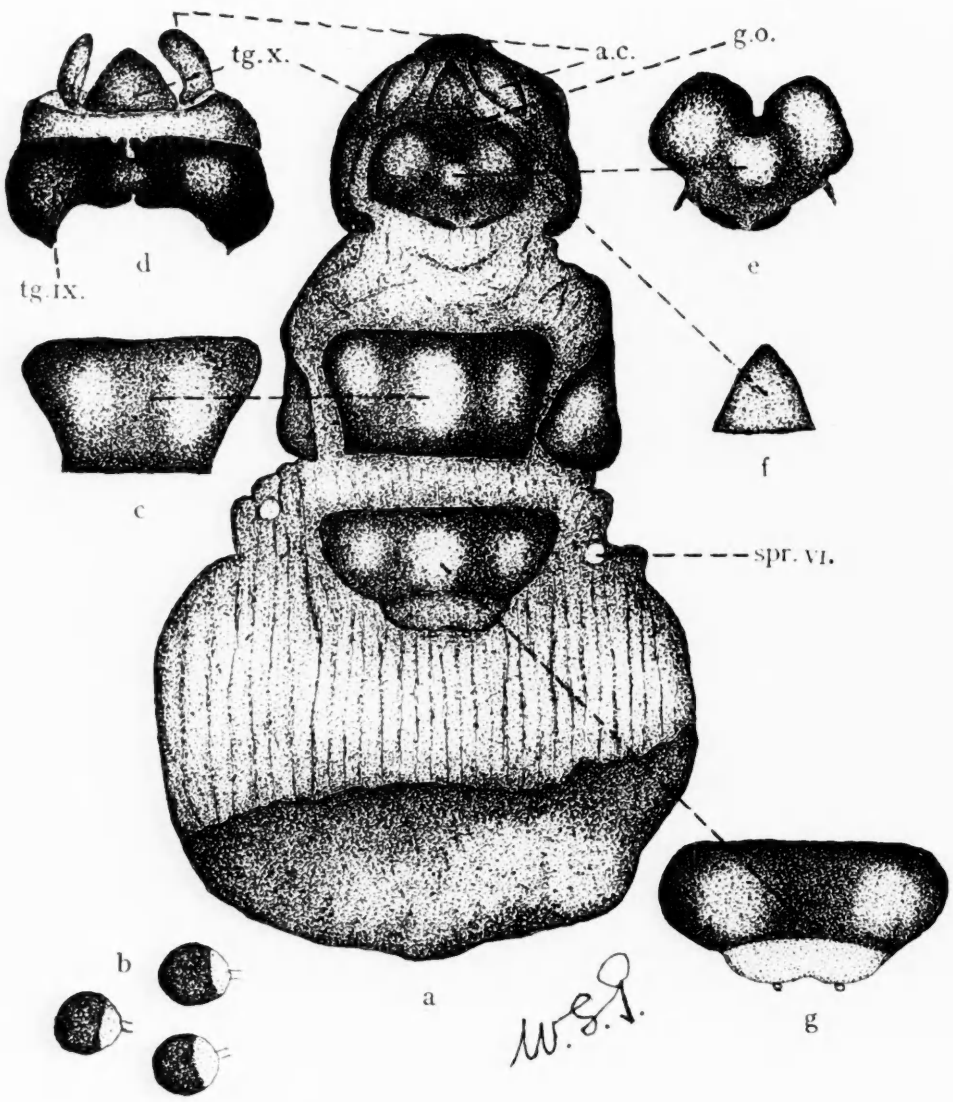

FIG. 3. *a.*—Ventral view of terminalia of ♀ *hominis* to show diagnostic characters ; *a.c.*—Anal cerci ; *g.o.*—Genital opening ; *spr. vi.*—Sixth spiracle ; the seventh spiracle is not seen ; *b.*—Spermathecae ; *c.*—Seventh sternum ; *d.*—Ninth tergum (tg. ix), tenth tergum (tg. x), and anal cerci (a.c.) ; *e.*—Ninth sternum ; *f.*—Tenth sternum ; *g.*—Sixth sternum (São Paulo, Brazil).

rest.) and *Bogeria* Austen, while the Central- and South-American species belong to *Cuterebra* Clark (sens. rest.), *Pseudogametes* Bischof and *Rogenhofera* Brauer.

As the terminalia of *Dermatobia* and *Cuterebra* are very fully illustrated, I do not propose giving any general description, but will confine myself strictly to the salient characters.

Dermatobia hominis L. Male Terminalia. The segmentation of the abdomen is as in other Muscidae described. The spiracles are located on the terga close to their ventro-lateral edges. Spiracle 6 lies in the membrane just anterior to tergum 6 (fig. 1), and spiracle 7 is located on the antero-lateral edge

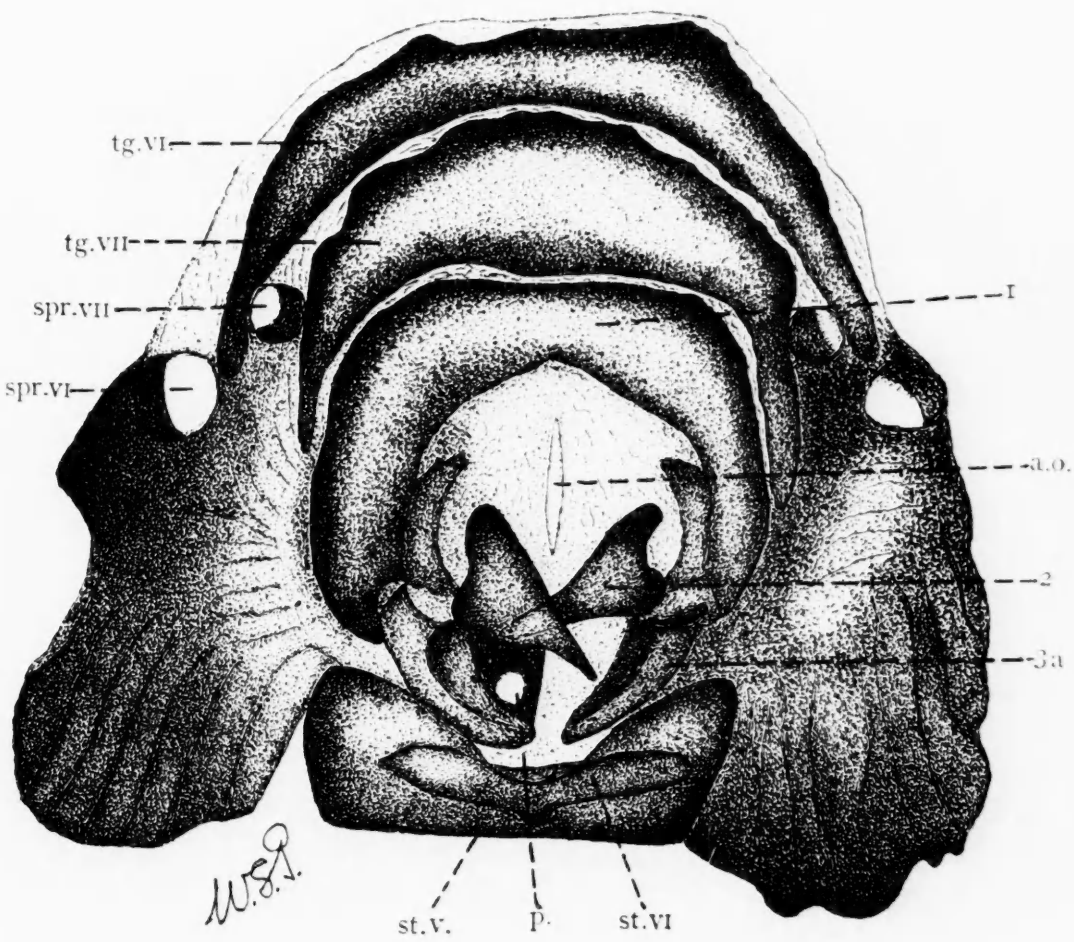

Fig. 4.—Ventral view of end of abdomen of ♂ *C. fontinella* showing relationships of parts; lettering as in fig. 1 (Ravalli Co., Montana, reared).

of tergum 7. Tergum 6 is a large rounded plate forming with tergum 7 the tip of the abdomen; tergum 7 is closely applied to it, but is shorter. Sternum 6 is a short, rather wide plate lying in the middle line above sternum 5 and concealed by it. Tergum 10 is a short, deeply incised plate, the anal cerci closing the incision. All these sclerites, as well as the anal cerci, ninth coxites and

phallosome, are illustrated in figs. 1 and 2 and do not call for a detailed description. The phallosome (fig. 2, *a*) is a long simple chitinous tube, bent ventrally and dilated distally, there being a narrow membraneous area between the two parts. The posterior paramere is a long, pointed, upstanding plate, and the anterior part is inconspicuous, being narrow and flat. To sum up, the fundamental characters of the ♂ terminalia are as follows :—(1) The segmentation

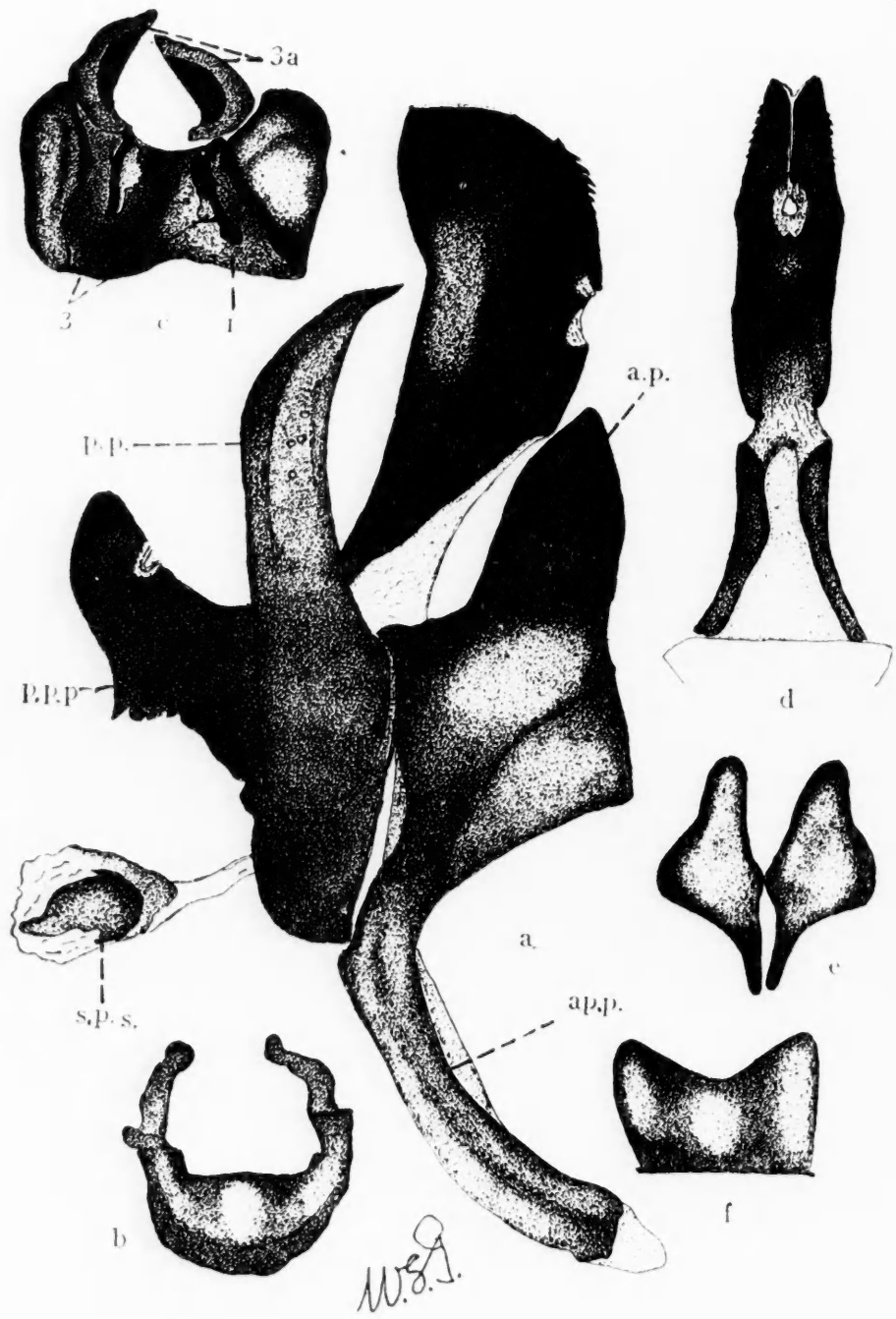

FIG. 5. *a*.—Phallosome and one paramere of *fontinella* in side view ; lettering as in fig. 2, *a* ; *b*.—Ninth tergo-sternum ; *c*.—Tenth tergum from within showing two parts of ninth coxites ; lettering as in fig. 2, *d* ; *d*.—Dorsal view of phallosome ; *e*.—Ventral view of anal cerci ; *f*.—Fifth sternum (Ravalli Co., Montana).

at the end of the abdomen is very complete for a muscid, the sixth tergum and sternum being well developed, the latter symmetrical and normal though reduced (cf. a similar condition in *Gasterophilus*). (2) The anal cerci are of a primitive structure and separate. (3) The distal segment of the ninth coxite is a simple lateral clasper.

FEMALE TERMINALIA. The ♀ terminalia are illustrated in fig. 3, and represent a simple ovipositor with the full complement of sclerites. The arthrodial membrane joining the terga is longer than that joining the sterna, thus permitting the ovipositor to be bent under the abdomen of insect carriers. The ninth sternum has a characteristic distal groove, which would permit the sclerite to be placed against a raised object, and would allow the eggs to be placed in a small heap. I have not been able to find any distinct anal opening ; the genital opening is a wide slit. The spermathecae (fig. 3, *b*) are small, round and partly pigmented.

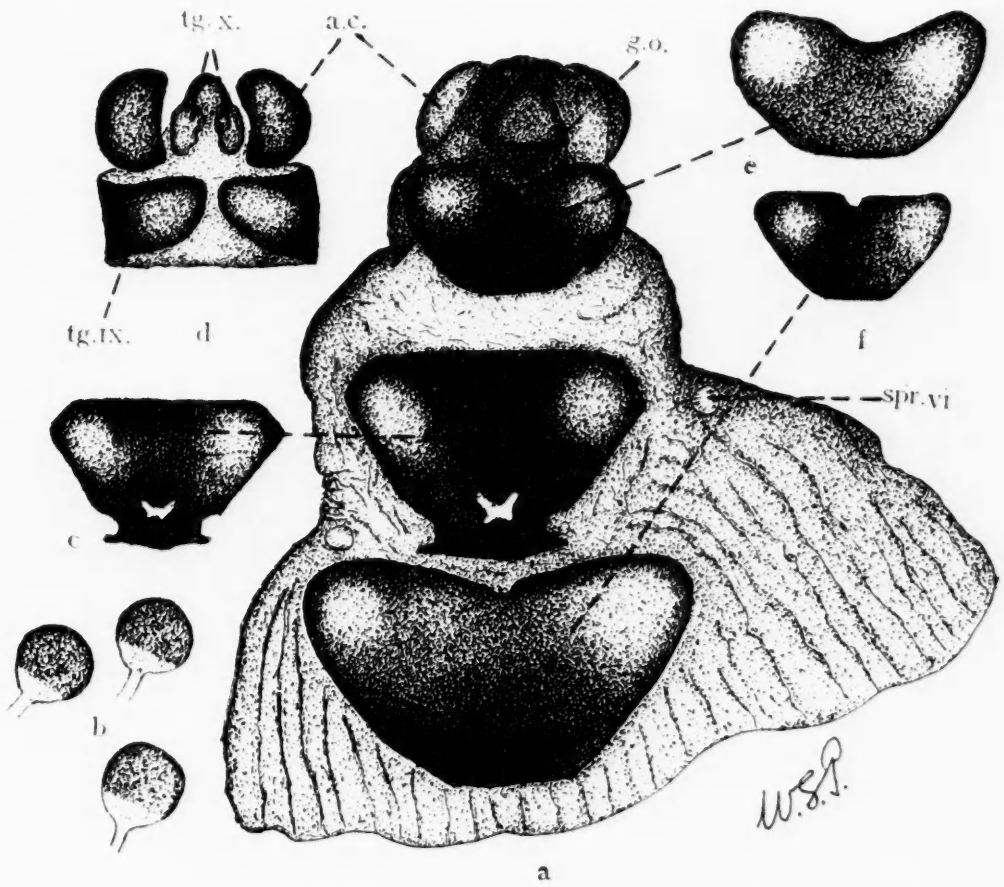

FIG. 6. *a*.—Ventral view of terminalia of ♀ *fontinella* to show diagnostic characters ; lettering as in fig. 3, *a* ; *b*.—Spermathecae ; *c*.—Seventh sternum ; *d*.—Ninth tergum (tg. ix), tenth tergum (tg. x) and anal cerci (a.c.) ; *e*.—Ninth sternum ; *f*.—Sixth sternum (Nicolum R. Hope, British Columbia).

Cuterebra fontinella Clark. MALE TERMINALIA. The ventral view of the end of the abdomen of the ♂ *fontinella* is illustrated in fig. 4 : it will be noted that it closely resembles that of *D. hominis* ; sternum 6 is here also well developed,

normal in position and not asymmetrical. As in *D. hominis*, the distal segments of the ninth coxites are typical lateral claspers. The phallosome is very similar in structure to that of *D. hominis*, except that the basal part of the distal end is more membraneous, and the membrane is wider where it separates the two halves of the dilated distal part, which are armed with teeth along their edges. The anterior paramere (fig. 5, *a*) is a short, bluntly pointed, stout, upstanding plate ; the posterior paramere (fig. 5, *a*) is very similar to that of *D. hominis*.

Female Terminalia. The ♀ terminalia of *fontinella* are illustrated in fig. 6. Here again the ovipositor has the full complement of sclerites, and in its general structure closely resembles that of *D. hominis*. The anal cerci are quite large and flap-like, and suggest that they are used to fix the eggs to some object which they clasp in the process ; I hardly think that it can be the hair of the

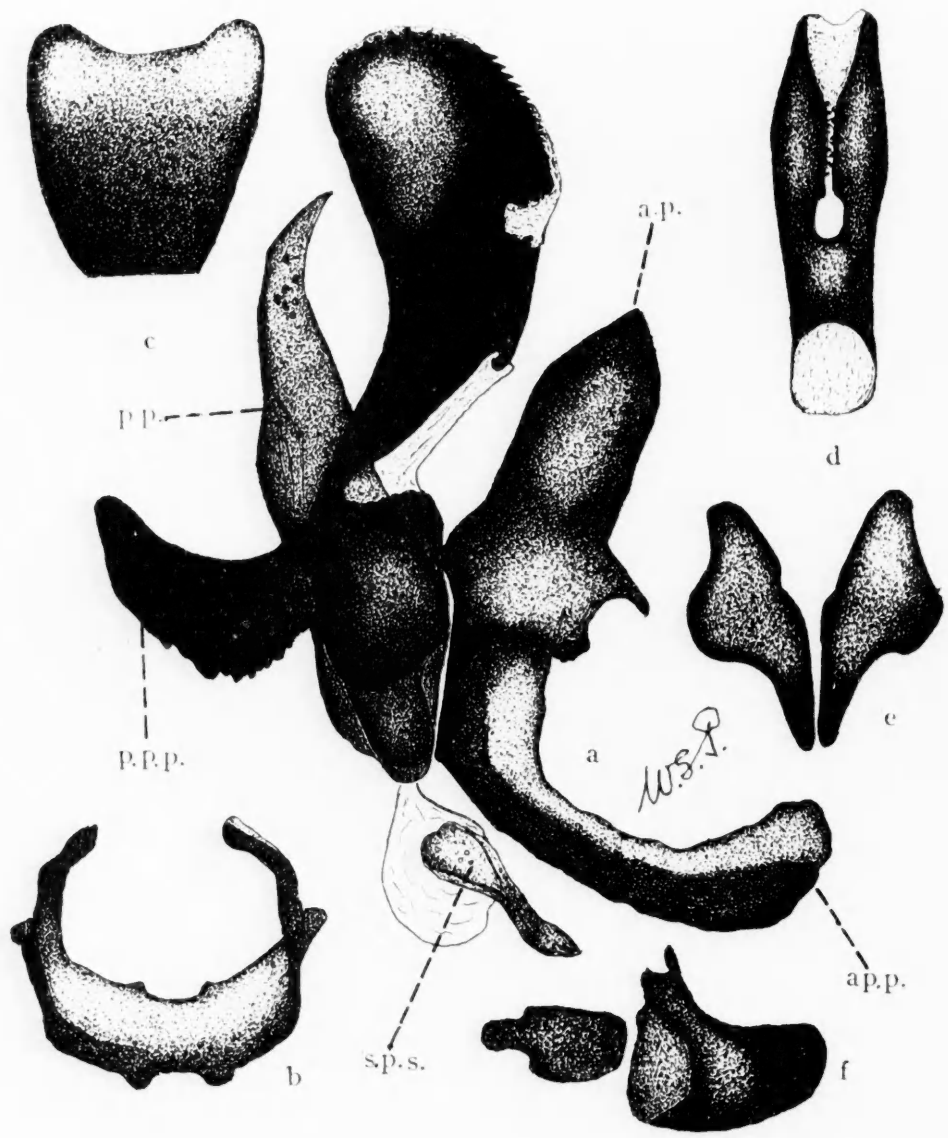

Fig. 7. *a*.—Phallosome and one paramere of *C. approximata* in side view ; lettering as in fig. 2, *a* ; *b*.—Ninth tergo-sternum ; *c*.—Fifth sternum ; *d*.—Dorsal view of phallosome ; *e*.—Ventral view of anal cerci ; *f*.—Two parts of paramere in side view (Ravalli Co., Montana).

host. Hadwen (1915) has described the egg of *fontinella*, and says that it resembles that of *Gasterophilus intestinalis* in shape, has an operculum, and a similar groove ; the surface of the egg is rough. The spermathecae of *fontinella* are exactly similar to those of *D. hominis*.

Cuterebra approximata Walker. MALE TERMINALIA. The ♂ terminalia of *C. approximata* are illustrated in fig. 7. On comparing the structure of the parts with that of the terminalia of *fontinella* it will be noted that they are exactly similar, the differences being of a minor character : the anal cerci of *approximata* have, for instance, broader distal ends than those of *fontinella*, and there is a slight difference in the structure of the phallosome and the posterior part of the paramere.

Cuterebra americana F. MALE TERMINALIA. The ♂ terminalia of *C. americana* are illustrated in fig. 8. Here again it will be noted that the parts

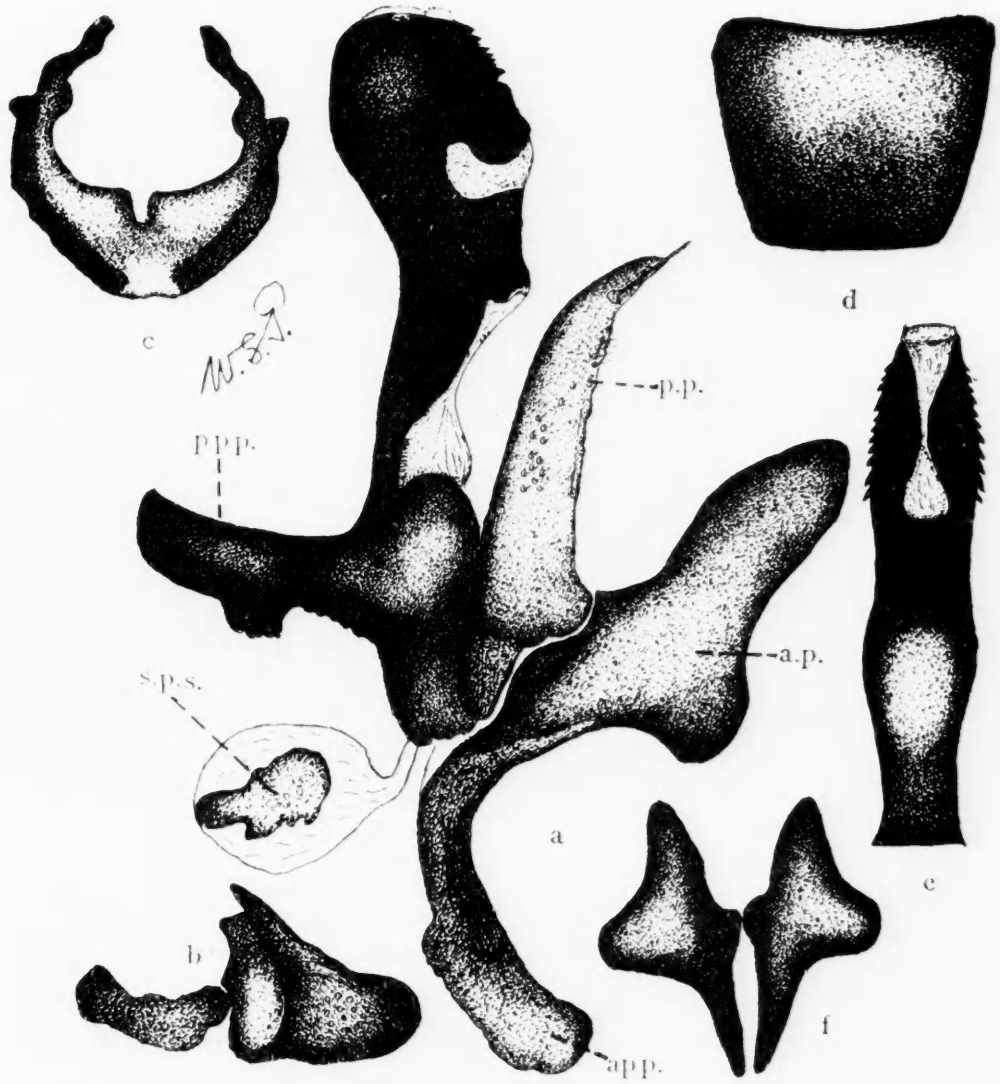

FIG. 8. *a*.—Phallosome and one paramere of *C. americana* in side view ; lettering as in fig. 2, *a* ; *b*.—Two parts of ninth coxite in side view ; *c*.—Ninth tergo-sternum ; *d*.—Fifth sternum *e*.—Dorsal view of phallosome ; *f*.—Ventral view of anal cerci (Castle Butte, Arizona, U.S.A.).

are almost exactly similar to those of *fontinella* and *approximata*, the differences being very small.

Cuterebra atrox Clark. MALE TERMINALIA. The ♂ terminalia of *C. atrox* are illustrated in fig. 9: it will be noted they are identical in structure with those of the preceding species. The posterior paramere in this species is very suggestive of that of *Hypoderma*.

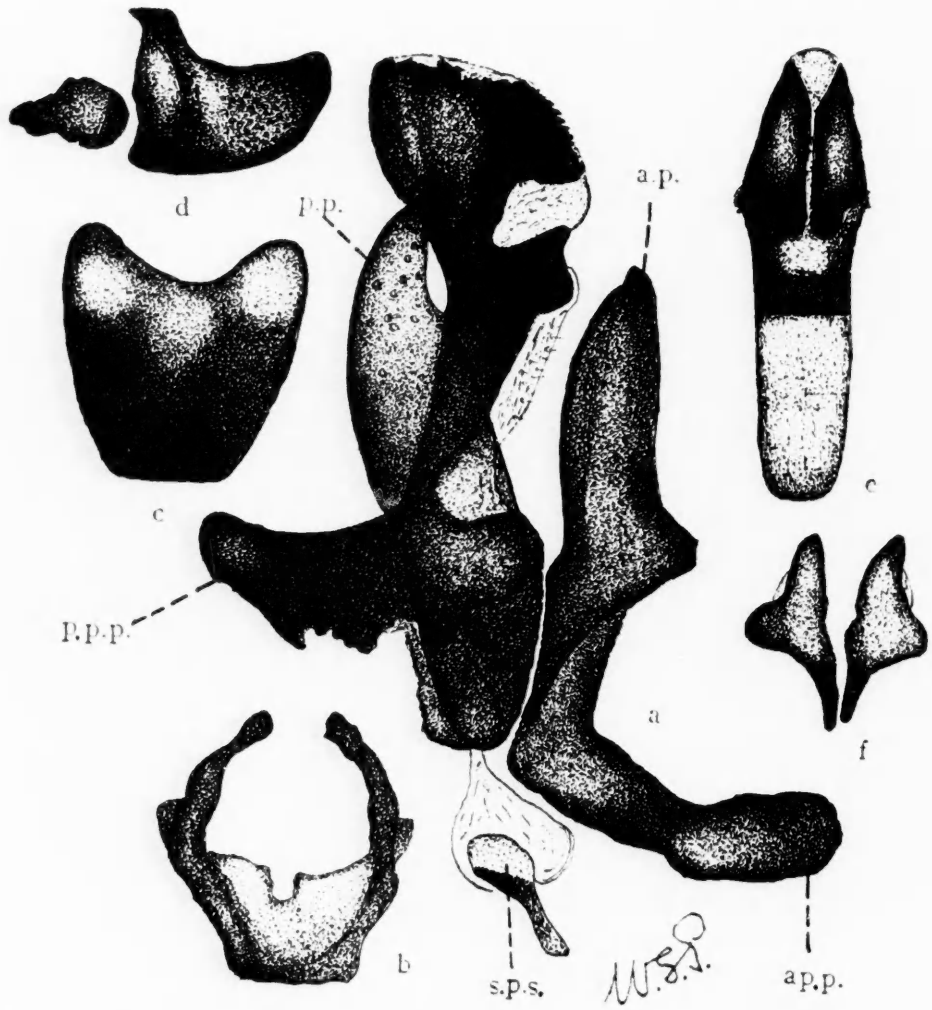

FIG. 9. *a*.—Phallosome and one paramere of *C. atrox* in side view; lettering as in fig. 2, *a*; *b*.—Ninth tergo-sternum; *c*.—Fifth sternum; *d*.—Two parts of ninth coxite in side view; *e*.—Dorsal view of phallosome; *f*.—Ventral view of anal cerci (Magdalena Mts., N. Mex., 8,500 feet, July 31st).

Cuterebra (Bogeria) species incert. MALE TERMINALIA. The ♂ terminalia of a species of *Bogeria* bred from a larva from the cottontail are illustrated in fig. 10; as the specimen was a teneral one Major Austen was unable to give it a name. It is hardly necessary to draw attention to the striking resemblance of

the parts of the terminalia to those of the species illustrated above ; they leave no doubt that it is a true *Cuterebra*.

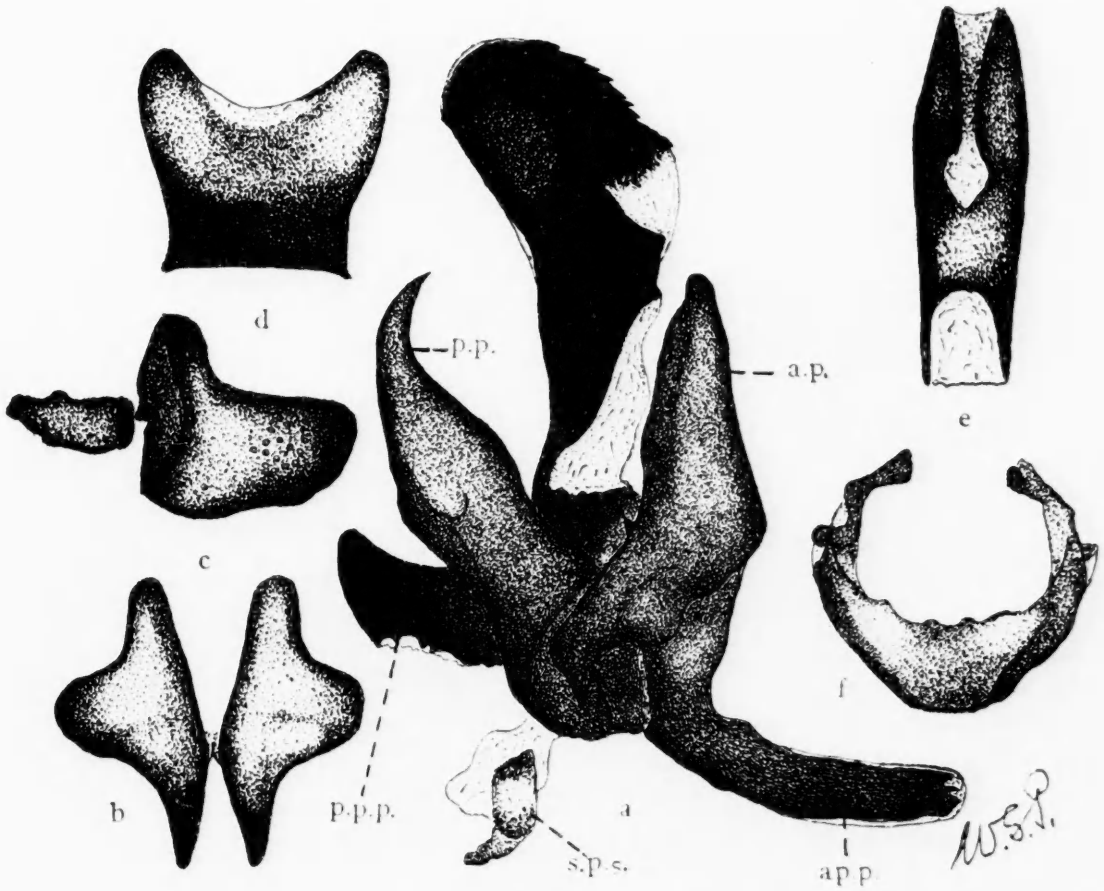

FIG. 10. *a*.—Phallosome and one paramere of a large, undetermined (teneral) species of *Bogeria* ; lettering as in fig. 2, *a* ; *b*.—Ventral view of anal cerci ; *c*.—Two parts of ninth coxite in side view ; *d*.—Fifth sternum ; *e*.—Dorsal view of phallosome ; *f*.—Ninth tergo-sternum (Yokum Valley, Cottontail).

Cuterebra (Bogeria) species incert. MALE TERMINALIA. The ♂ terminalia of a small species of *Bogeria* are illustrated in fig. 11. This species from the Blue Nose Peak, West Fork, Ravalli County, Montana, is another typical *Cuterebra*. The parts are structurally similar to those of the other five species illustrated.

A comparative study of the illustrations of the parts of the terminalia of these species will demonstrate the fact that, as the parts are very similar and the differences very small, it would be difficult to use these characters for the identification of the species without dissection. The only characters which might be of use without dissection are the anal cerci, which differ in length and width in the species.

THE SYSTEMATIC POSITION OF *Dermatobia* AND *Cuterebra*. Before considering the systematic position of these two genera, it is necessary to draw attention to the fundamental characters of the terminalia. The ♂ terminalia of both genera are relatively small and inconspicuous. The sixth tergum and

sternum are both well developed, especially the latter, which is normal in structure and position, there being no asymmetry. The two anal cerci are distinct and elongated, and grasp the end of the abdomen. The distal segments of the ninth coxites are typical, simple lateral claspers, just as in *Gasterophilus* and *Hypoderma*. The phallosome in both is long and is a simple tube, that of

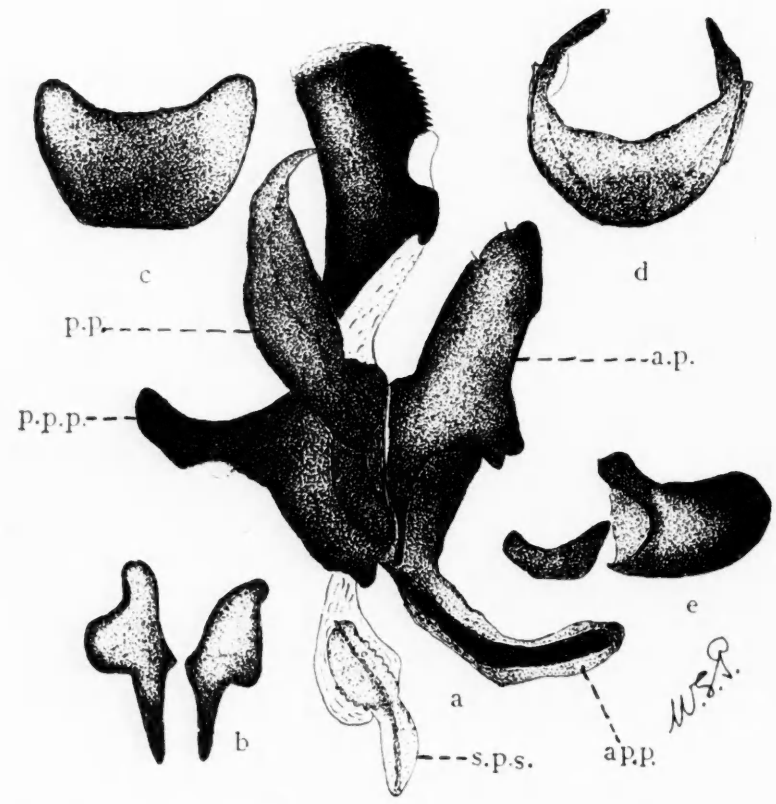

FIG. 11. *a*.—Phallosome and one paramere of a small, undetermined species of *Bogeria*; lettering as in fig. 2, *a*; *b*.—Ventral view of anal cerci; *c*.—Fifth sternum; *d*.—Ninth tergosternum; *e*.—Two parts of ninth coxite in side view (Blue Nose Peak, West Fork, Ravalli Co., Montana).

Dermatobia being perhaps nearer the ancestral type. All these primitive characters clearly point to these flies being very ancient members of the family Mucidae. The ♀ terminalia, too, exhibit primitive characters, all the sclerites of a simple, short ovipositor being present. Each is adapted to the egg-laying habit; that of *Hypoderma* allows the ovipositor to be bent under the body of the insect carrier, while that of *fontinella* permits the egg to be laid on some object (side of wall of burrow) in the habitat of the host. In this case it is not known whether the larva leaves the egg of its own accord, nor how it reaches its destination in the skin of the host (whether directly or by the alimentary canal). Is *Dermatobia* related to *Cuterebra*? Although it has been assumed that it is, I know of no proof for this assumption. From a comparative study of the terminalia I have no hesitation in saying that *Dermatobia* is clearly related to *Cuterebra*, and should be placed in a group with it. In both genera the phallosome is fundamentally of similar structure; the posterior paramere in each is also structurally

similar, but the anterior part in *Dermatobia* is in an undeveloped condition. The ♀ terminalia of both are strikingly similar, and the spermathecae in both are almost identical. There can, therefore, be no doubt that the two genera are closely related and probably have had a common origin. It is probable that when the terminalia of some of the Central- and South-American species of *Cuterebra* are studied a link between the two may be discovered.

With regard to *Cuterebra*, this study has demonstrated most convincingly that the four species *americana*, *approximata*, *atrox* and *fontinella* are not only very closely related to each other but are also closely related to the two species of *Bogeria* studied. It is only necessary to refer to the drawings of the ♂ terminalia of the six species to appreciate the truth of this statement. The ♂ terminalia are, in fact, so similar that it is difficult to find characters which one could use to separate the species. These microscopic characters could only be used with success after dissecting the parts. I may here point out that, except for the phallosome of *approximata*, all the parts of each species are drawn to the same magnification. I have no doubt that when the terminalia of other species are illustrated—and even those of the South-American *Pseudogametes* and *Rogenhofera*—it will be found that they are structurally similar. It is clear to me, therefore, that the systematist has two possible ways of classifying these flies, either (1) by making a genus for each species, or (2) by placing all in the genus *Cuterebra*. I have no doubt that the second is the correct and only solution to this question. Whether it will be possible for me to study the terminalia of *Pseudogametes* and *Rogenhofera* seems very doubtful, as these species are rare. I note, however, that Lutz (1918), in describing *Pseudogametes semiater* Wied., says : ' It was discovered in Petropolis by Mr. Foetterle, who makes a special study of Lepidoptera. Over a hundred specimens, almost all males, were collected in several years ; with only two or three exceptions, they all occupied the same small section of the trunk of the same tree, 3–4 meters from the ground. They were found in the summer months only and chiefly in February. The first appeared almost exactly at 9 o'clock in the morning ; they settled on the bark remaining there for hours on stretch and were almost always solitary, never in large numbers.'

I have no idea where these hundred ♂♂ are preserved, but I hope that, if any reader knows, he will try to get me a few for the critical study of the ♂ terminalia.

It is next necessary to consider the question of the true systematic position of these genera. A comparative study of the ♂ terminalia of *Dermatobia* and *Cuterebra* with those of *Hypoderma* has led to the conclusion that they are related. The phallosome of *Hypoderma* is very similar to that of *Cuterebra*, the only real difference being that it is more membraneous distally. The two parts of the paramere, too, are strikingly alike. And both are dermal parasites. I propose, therefore, to classify *Dermatobia* and *Cuterebra* as genera in the subfamily Hypodermatinae in the family Muscidae. I believe that *Dermatobia* represents the primitive member of this large group, that *Cuterebra* occupies

an intermediate position, and that *Hypoderma* is geologically a more recent form.

In my next few papers in this series I shall continue the comparative study of the terminalia of the species of *Glossina*. Later I hope to illustrate the terminalia of *Cephenemyia*, *Oestrus*, *Cephalopsis* and *Cobboldia*, and to discuss the systematic position of these genera, and still later to describe the terminalia of some Hippoboscinae. I shall always be glad to get any adults of the parasitic Diptera, and I hope that my readers will help in this direction.

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(To be continued)

RESEARCHES ON BLACKWATER FEVER IN GREECE

III.—A NEW PHOTO-NEPHELOMETRIC METHOD FOR THE QUANTITATIVE ESTIMATION OF MINUTE AMOUNTS OF QUININE IN FAECES AND BODY FLUIDS

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These studies were undertaken, during the course of work on blackwater fever in Greece, in an attempt to throw some light on the action of quinine in this disease after blockade of the reticulo-endothelial system with thorotrast or electro-colloidal copper (Heyden); on the relation of this blockade to the effectiveness of quinine in clearing the blood of malaria parasites; and on the question whether blockade has any effect upon the blood levels and upon the rate of excretion of quinine.

Before any of these problems could be attacked, it was necessary to develop some practicable means of estimating quinine in blood and urine, and if possible in faeces. It also seemed desirable to use some method for ascertaining to what extent the blockade of the reticulo-endothelial system had become effective, since it would appear to be impossible to draw conclusions (as some have done) from such blockade work unless a measure of the degree to which blockage had progressed was available. To estimate this we decided to measure the rate of cell-respiration of minced liver and spleen in blockaded and non-blockaded cases, using the method of Fenn (1927), as recently modified by Ramsey and Warren (1930), and to check this by the indicator method of Jungeblut and Berlot (1926). As it was only possible to carry out these procedures in cases where an autopsy was made, we endeavoured to correlate the cell-respiration rate in blocked and unblocked cases with the level of bilirubin in the blood. The preliminary work was done on rabbits and has already in part been reported (Foy and Kondi, 1935*a*). A full account of these researches will be given later; here it is only desired to call attention to what we believe to be a new method for estimating minute amounts of quinine in 1 c.cm. of urine (which can also be made applicable to faeces), and to certain improvements which we have introduced into the methods for blood quinine.

Since Ramsden and Lipkin (1918) described their laborious and more-or-less impracticable methods for blood and urine quinine estimation, little or no real advance has been made in the study of blood quinine levels and their

correlation with the excretion of the alkaloid by the renal and alimentary routes. A certain amount of work has been carried out on blood quinine levels alone, after the administration of the drug in graded doses by different channels (St. John, 1932 ; Karamchandani, 1933 ; Mattei, 1930 ; Friesz and Hallay, 1930 ; Binet and Fabre, 1931 ; Vedder and Masen, 1931 ; Chopra, Roy and Das Gupta, 1934 ; etc.) ; but little attempt has been made to relate the wide variations with differential excretion rates.

Acton and King (1921) modified Ramsden and Lipkin's method and reduced the time and labour needed to make the estimations, but still left much to be desired both as to practicability and sensitivity. Roy (1926) increased the sensitiveness of the colorimetric methods by introducing a modified Wagner's reagent indicator for blood quinine ; and later Vedder and Masen (1931) still further improved the colorimetric method by introducing gum-ghatti and a potassium bismuth iodide indicator, claiming that the gum-ghatti stabilized the colour of their indicator, and that with this indicator the large number of standards necessary for use with Roy's reagent were not needed. From our own spectrophotometric work we have come to the conclusion that the instability of the colour in both these colorimetric methods render them almost worthless, at least for quantitative work aspiring to any degree of accuracy. In view of the uncertainties of the colorimetric methods, we decided to modify Vedder and Masen's nephelometric method for blood quinine, and we have used our modification throughout this work. For the estimation of quinine in urine we have developed what we think to be a hitherto undescribed method, which with some modification can be applied to faeces. The sensitivity and accuracy of this method, as will be seen from Tables I and II, is almost all that can be desired,

TABLE I

Quantitative recovery of quinine added to urine

	Quantity of quinine added to urine	Quantity of quinine recovered
(*a*)	40 mg. per litre	41 mg. per litre
(*b*)	40 mg. ,,	39 mg. ,,
(*c*)	100 mg. ,,	100 mg. ,,
(*d*)	100 mg. ,,	100 mg. ,,
(*e*)	20 mg. ,,	20 mg. ,,
(*f*)	20 mg. ,,	20·8 mg. ,,
(*g*)	10 mg. ,,	9·7 mg. ,,
(*h*)	10 mg. ,,	10·2 mg. ,,

and so far as we have been able to discover reacts with no other substance present in normal or pathological urines ;* moreover, the faeces that we have

* A positive reaction is given with atropine, morphine and strychnine, and no doubt with all other alkaloids, but these can generally be excluded.

so far had occasion to analyse have never given a positive reaction except in cases where quinine has been given, but we do not claim for faeces that a substance will not be found in either normal or pathological stools that may not give a positive reaction, considering the great variety of food-stuffs eaten. We

TABLE II

Quantitative recovery of quinine added to blood

	Quantity of quinine added to blood	Quantity of quinine recovered
(*a*)	0·015 mg. in 3 c.cm.	0·015 mg. in 3 c.cm.
(*b*)	0·015 mg. „ „	0·014 mg. „ „
(*c*)	0·007 mg. „ „	0·007 mg. „ „
(*d*)	0·007 mg. „ „	0·007 mg. „ „
(*e*)	0·03 mg. „ „	0·029 mg. „ „
(*f*)	0·03 mg. „ „	0·028 mg. „ „
(*g*)	0·03 mg. „ „	0·029 mg. „ „

also used the same method for rabbit-blood, urine and faeces. The sensitivity of the test described is limited to about 0·5 mg. per litre. In the case of Mayer's and Wagner's reagents the limit of sensitivity is about 2–4 mg. per litre, whilst Roy's reagent (Roy, 1926) is stated to be in the neighbourhood of 0·05 mg. per litre (1 in 20 million), but the instability of this indicator and the necessity for preparing a large number of standards make it, for all practical purposes, useless.

Throughout our work we have used the spectrophotometer of Pulfrich, with a special nephelometric attachment supplied to us by Carl Zeiss, and fitted with a standardized interchangeable turbidity prism of known value. The advantages of the use of such an instrument over the usual type of nephelometer are several. It enables ' absolute ' turbidity values to be obtained. Further, in dealing with high concentrations of quinine, the Tyndall effect will be masked by light-absorption, and it will therefore be difficult with the ordinary type of nephelometer to decide exactly what the reading is without resorting to methods of dilution, and the construction of special concentration-curves for use with strong solutions and their concomitant standards. With the instrument that we have used, standards are unnecessary, and it is perfectly easy to decide whether the reflected light is in any way being interfered with by light-absorption on account of the great turbidity of the solution. In addition, the fluorescence emitted by quinine solutions makes it necessary to eliminate all light-waves below 6,000 A°, which we have effected by using a filter in the nephelometric light and by transmitting the reflected light so obtained through a second filter on the spectrophotometer, cutting out all the waves below 6,000 A°.

With the silico-tungstic acid which we use for producing the turbidity, we

have found that a temperature of 30°–40° C. is ideal for maintaining perfect suspension of the particles in the fluid ; it is therefore desirable to fill the water-chamber of the instrument with distilled water at this temperature, and if necessary to keep it in circulation in cases where large numbers of estimations are to be made.

Needless to say, the methods being nephelometric, the utmost precautions must be taken to ensure that no extraneous particles gain entrance to the final solutions to impair accuracy of the work. If the spectrophotometer with its nephelometric attachment is not available, the ordinary nephelometer of the Kober type may be substituted ; but with such instruments, of course, standard solutions are necessary, and, since these must not differ from the unknown by more than 20 per cent., it is generally necessary to prepare a whole series—especially when urine and faeces are to be dealt with, where the quinine content is likely to vary within very wide limits. However, with practice, moderate accuracy can be attained. With these types of nephelometers, the precaution of setting the light-source must always be taken by placing the standard solution in both cups, setting both plungers at, say, 15 mm. and balancing the intensity of light in both sides of the light-field in the eye-piece by adjusting the light-source. Care must also be taken to see that chipped plungers are not used, otherwise this will prevent anything like accurate readings from being made. If the instrument is fitted with octagonal plungers, chipping is very hard to prevent, and the utmost care must be taken in handling them. It is better to use round plungers, but even here care must be exercised to see that the bottoms are not damaged. In estimating the quantity of quinine in body fluids the amount of the final solution available is generally small—3–6 c.cm. ; it is therefore desirable to use micro-cups and plungers, thus enabling some of the solution to be used for the purpose of washing the cups and plungers—a very important precaution in nephelometric work.

With the Kober type of the nephelometer no concentration-curve is necessary, and the usual colorimetric formula may be used for calculating the results, as will be shown below. We have sometimes used, with moderate success, a Klett nephelometer-colorimeter, which is built on the Kober principle ; and, although the ease of working, range and accuracy are not to be compared with the Pulfrich instrument, quite good results can be obtained if the usual precautions necessary for nephelometric work are adhered to (Yoe, 1929).

URINE

Discussion

The amount of urine taken for the extraction will depend upon the quantity of quinine that it is expected to contain : if large amounts of quinine are suspected, then only a small amount of urine need be taken, and *vice versa*. The reason for varying the amounts of urine taken is that, if too large amounts are used, the process of extraction will be long and tedious and the amount of

ether required large ; it is well, therefore, to take the smallest amount of urine compatible with accuracy. The quantity of quinine excreted in the urine passed during the 48 hours following the taking of the drug is subject to very wide variations in different individuals and in the same individual at different times. This is illustrated in Tables III, IIIA and IIIB and the accompanying graphs. The figures are taken from the same individual after doses of the same quantity of quinine sulphate (400 mg.). The times of passing the various specimens of urine are noted, together with their amounts, their quinine content and the calculated quinine value per litre. From these Tables it will be seen that the quantity of drug excreted by the renal route in the same individual after a similar dose of quinine ranged from 19 mg. per litre to 33 mg. per litre. It will be noted that in all cases the maximum excretion occurred after 9–10 hours ; we have found this to hold for doses of different sizes, which would make it seem that the period needed to reach the maximum rate of excretion is not dependent upon the size of the dose, but upon other, yet unknown, factors, as has been shown to exist for blood quinine intravenously injected (Gehlen, 1933). At present we are unable to make any statement as to the quantity of quinine likely to be found in a given specimen of urine, but it would appear roughly to vary between 15 mg. per litre and 40 mg. per litre after a 400 mg. dose. At

TABLE III

Showing the amount of quinine excreted in the urine at varying intervals, after a 400 mg. dose of quinine sulphate

Specimen of urine	Amount of specimen	Time elapsed from taking quinine to passing urine	Amount of quinine in specimen	Equivalent amount of quinine per litre
1st	164 c.cm.	2 hours	1·4 mg.	8·5 mg. per litre
2nd	154 c.cm.	5 ,,	6·1 mg.	39·6 mg. ,,
3rd	78 c.cm.	8 ,,	5·0 mg.	64·6 mg. ,,
4th	152 c.cm.	10 ,,	11·0 mg.	72·0 mg. ,,
5th	122 c.cm.	13 ,,	3·8 mg.	31·0 mg. ,,
6th	214 c.cm.	24 ,,	4·5 mg.	20·0 mg. ,,
7th	150 c.cm.	27 ,,	1·5 mg.	10·0 mg. ,,
8th	80 c.cm.	30 ,,	0·5 mg.	6·2 mg. ,,
9th	100 c.cm.	34 ,,	0·5 mg.	5·0 mg. ,,
10th	250 c.cm.	38 ,,	0·7 mg.	2·8 mg. ,,
11th	180 c.cm.	42 ,,	Traces	
	1,644 c.cm. total	Total amount of quinine excreted per litre in the 42 hours = 21·0 mg.	35·0 mg. total in 1,646 c.cm.	

TABLE IIIA

Showing the amount of quinine excreted in the urine passed at varying intervals, after a 400 mg. dose of quinine sulphate

Specimen of urine	Amount of specimen	Time elapsed from taking quinine to passing urine	Amount of quinine in specimen	Equivalent amount of quinine per litre
1st	150 c.cm.	4 hours	4·95 mg.	33·0 mg. per litre
2nd	293 c.cm.	10 ,,	11·1 mg.	38·0 mg. ,,
3rd	150 c.cm.	13 ,,	3·15 mg.	21·0 mg. ,,
4th	180 c.cm.	23 ,,	5·76 mg.	32·0 mg. ,,
5th	180 c.cm.	26 ,,	1·10 mg.	6·2 mg. ,,
6th	230 c.cm.	32 ,,	0·72 mg.	0·7 mg. ,,
7th	220 c.cm.	35 ,,	Traces	Traces
	1,402 c.cm. total	Total amount per litre excreted in 35 hours = 19·1 mg.	26·78 mg. total	

TABLE IIIB

Showing the amount of quinine excreted in the urine at varying intervals, after a 400 mg. dose of quinine sulphate

Specimen of urine	Amount of specimen	Time elapsed from taking quinine to passing urine	Amount of quinine in specimen	Equivalent amount of quinine per litre
1st	180 c.cm.	4 hours	11·6 mg.	64·2 mg. per litre
2nd	220 c.cm.	10 ,,	24·8 mg.	113·0 mg. ,,
3rd	170 c.cm.	18 ,,	7·6 m.g.	45·0 mg. ,,
4th	115 c.cm.	21 ,,	3·0 mg.	26·0 mg. ,,
5th	170 c.cm.	26 ,,	2·9 mg.	17·0 mg. ,,
6th	200 c.cm.	36 ,,	1·5 mg.	7·5 mg. ,,
7th	370 c.cm.	45 ,,	1·7 mg.	4·6 mg. ,,
8th	154 c.cm.	51 ,,	Negative	Negative
	1,579 c.cm. total	Total amount per litre excreted in 45 hours = 33·5 mg.	53·1 mg. total	

present we cannot make any statement upon the precise reason for these wide variations in the amount excreted after a similar dose ; perhaps variations in the solubility of the salt taken may have some effect, as well as the length of time the drug remains in the alimentary canal. Without at the moment making any definite ruling on the matter, it would seem that, after a 400 mg. dose of quinine sulphate in an individual of 60 kilos., the amount of quinine recoverable from the faeces may amount to as much as 300 mg., that in the urine between 15 mg. and 40 mg. per litre. Since the blood quinine level varies between 2 mg. and 10 mg. per litre, the question of what becomes of the remainder of the absorbed quinine is a matter of some interest. Dawson and Garbade (1930) have shown that the liver is responsible for the conversion of quinine into a non-malaria toxic compound quitenine, and it is possible that the balance may be accounted for here. In any case, it would appear that the bulk of the quinine taken by the oral route is, in a certain number of cases at least, excreted in the faeces apparently unchanged. In the case of intramuscular quinine, it would appear that in most cases the bulk of the drug remains at the site of the injection, to give rise in some cases later to quinine abscesses. With regard to the fate of intravenous quinine, we can make no statement at the moment.

As a general guide only, it may be stated that, if the amount of quinine is expected to be less than 20 mg. per litre, then 2 c.cm. of urine should be taken for extraction ; and if more than 20 mg. per litre, then 1 c.cm. should be used. It is important that the turbidity produced when the silico-tungstic acid is added should be in the form of a fine suspension, and not in the form of gross flocculations, as will happen if the amount of quinine present is too large ; hence, in judging the amount of urine to use, and the quantity of acid in which it should be taken up (see later), this fact should be borne in mind. If only the 24-hour whole specimen is to be examined, then it will generally be safe to use 1 c.cm. of urine, taking it up in 10 c.cm. of 0·5 N. HCl acid. Having decided the amount of urine to take then proceed as follows :—

Method

To the whole specimen of urine a few drops of concentrated ammonium hydroxide are added until the reaction is faintly alkaline to litmus ; too much alkali is to be avoided, as it causes a dense precipitate to form and hinders the process of extraction, but if there is too little the final filtrate will be tinted red. One or two c.cm. of this alkalized urine are taken and pipetted into a conical separating funnel (the more conical and less round the funnel the better, as it facilitates the separation of the layers of ether and urine), about 10 c.cm. of pure methylated ether are added, and the whole is vigorously shaken for five minutes. During the shaking it is advisable to lift the stopper from the funnel once or twice to allow the ether vapour to escape, otherwise the stopper may fly out unexpectedly and some loss of the contents may result. After shaking, place aside for a few minutes to permit the ether and urine to separate. When this has taken place, carefully open the tap and draw off the bottom layer of

urine, allowing a little ether to pass out with it. This urine is collected carefully for the next extraction that is to follow. The urine having been drawn off, the ether in the funnel is *whirled* round so as to dislodge any fatty droplets and débris that are adhering to the walls of the funnel ; shaking will not dislodge these. After several seconds of whirling whitish droplets will be seen swirling round in the ether ; these are allowed to settle to the bottom of the funnel and are then drawn off and added to the first lot of urine already collected. The whirling is again carried out and the droplets drawn off, and the process is repeated until no more droplets or débris collect at the bottom of the funnel, and the ether is clear. When this point has been reached, the ether is poured out through the *top* of the funnel (not through the tap, as this will contain a great quantity of the fatty particles and débris which will contaminate the clean ether) into a 100 c.cm. Erlenmeyer flask. The urine and fatty droplets that have been collected are now returned to the funnel, a fresh 10 c.cm. of ether is added, and the same process as before is repeated, the urines being separated, poured off and collected, and the clear ether added to the first lot in the Erlenmeyer flask. This is repeated three times, and all the ethers are collected together in the Erlenmeyer flask.

The collected ethers, about 30 c.cm., are now evaporated to complete dryness on a boiling water-bath ; 5 c.cm. or 10 c.cm.* of 0·5N. HCl acid are now added, and the flask is returned to the water-bath for five minutes to facilitate the solution of the quinine ; while still hot, filter through a Whatman 42 paper or some other high-grade hairless and dust-free paper. The filtrate, since it is for nephelometric examination, should be crystal-clear and free from all dust and extraneous matter. Cool under the tap. Select a test-tube that is free from the ' drawing lines ' that are often produced during the manufacture, or any other faults, such as bubbles in the glass, that are likely to set up light-reflection. (A number of such test-tubes should be sorted out and kept for the special purpose of nephelometric work alone.) Into one of these test-tubes place 3 c.cm. or 6 c.cm. (according to the amount of acid that was used) of the crystal-clear filtrate, and add to it 0·06 c.cm. or 0·12 c.cm. of 10 per cent. silico-tungstic acid (the smaller amount if 3 c.cm. is taken, the larger if 6 c.cm. of the filtrate is used). Shake the tube well and return to the boiling water-bath for 5 minutes. After this heating, plunge the tubes into cold water, and thoroughly cool under the tap. When quite cool, shake and place in the nephelometer and read.

Setting the nephelometer, and calculation

Fill the nephelometer chamber with water, washing it out once to ensure that no dust is present. Place the tube with the test solution in the holder and turn on the light. For tubes having an inside diameter of 10–20 mm., the illumination stop at the back of the instrument must be pulled out to its fullest

* 5 c.cm., if the amount of quinine expected is small ; 10 c.cm., if larger amounts of quinine are suspected.

point, so as to produce a wedge-shaped beam of light. There should be sufficient fluid in the tubes entirely to envelope the light-beam. Now, by means of the hinged eye-piece magnifier attached to the Pulfrich, determine, while observing through the eye-piece, whether the tube has any faults, such as scratches or bubbles, and turn the tube, while still observing through the eye-piece, until a spot is found that is free from blemishes. The filter cutting out light-waves below 6,000 A° units should be turned into the light, and a similar filter (one having a transmission maximum of 5,300 A°) should be used in the spectrophotometer, in order to compensate for fluorescence.

Set both drum-heads at 100 per cent., and, while observing through the eye-piece, turn the metallic disc containing the frosted screens till one is found that produces the nearest equality of light on the two sides of the light-field as seen in the eye-piece.

The indicator drums should be turned slowly, especially when the point of equality is being reached ; and the readings obtained should not differ by more than at the most 1 per cent. Three readings at least should be taken, and the mean struck. From these readings the relative opacity (RO) is calculated as follows :—When the ~~right~~ left-hand drum-head is set at 100 per cent., indicating that the unknown has a lesser turbidity than the frosted screen, the RO is calculated according to the formula

$$RO = 100 \times \frac{100}{\text{Readings}}$$

Example

$$RO = 100 \times \frac{100}{50} = 200$$

where 50 was the average of the three readings taken.

When the ~~left~~ right-hand drum-head is set at 100, indicating that the unknown has a greater turbidity than the frosted glass screen, the RO is represented by the readings on the ~~right~~ left-hand drum directly, and no calculation is needed. Since the turbidity value of the frosted glass screen was unknown and arbitrary, its value must be found in order to determine the absolute turbidity (AO). This AO is found by removing the test solution from the nephelometer holder and by placing the standard glass prism of known value in its place. Since the turbidity value of the prism is known and engraved on it by the manufacturers, the value for the screen and therefore for the test solution can readily be calculated as follows.

Leaving the same screen in place as was used for the unknown fluid, and the same wedge-shaped light-beam, with fluorescence filter, if used, in position, set both drum-heads again at 100. Observe which side of the light-field is the darker and set this drum-head at 100. (It may not be the same as for the unknown fluid.) Now rotate the other drum-head till the two light-fields exactly match ;

take three readings, strike the mean of the three, and calculate the AO according to the following formula :—

$$AO = \frac{RO}{H} \times D \times T$$

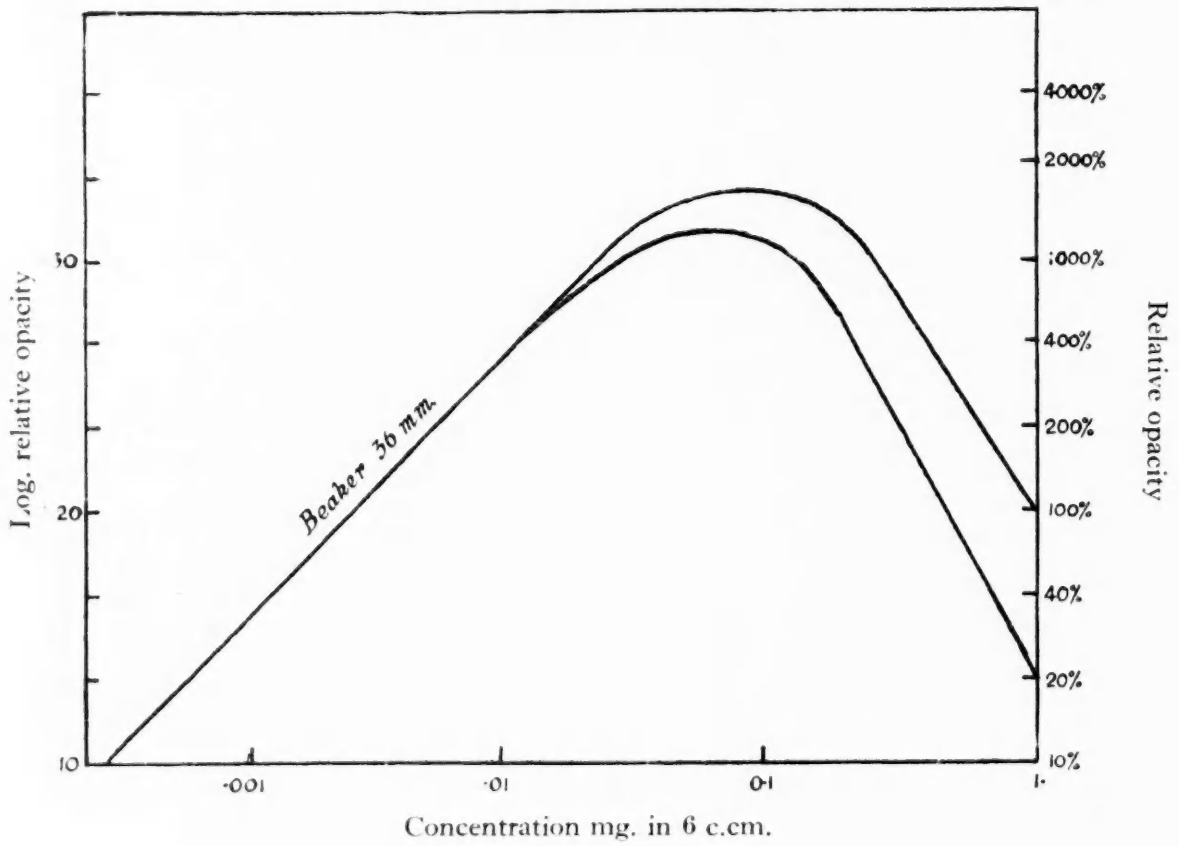
where AO = Absolute turbidity.
,, RO = Relative ,, (from previous calculation).
,, H = Readings obtained when the standard turbidity prism was in place.
,, T = Turbidity value of the standard prism as engraved upon it.
,, D = The factor for test-tubes having a diameter of 10–20 mm. with wedge-shaped light-beam which is always 1, and can be neglected.

Example $AO = \frac{200}{50} \times 0{\cdot}012 \times 1 = 0{\cdot}048$

On a previously constructed concentration-curve, this AO value is read off. In making up the concentration-curve the AO are plotted as ordinates, and the concentration as abscissae. In constructing this concentration-curve it is well to bear in mind that the quinine in the unknown solution was the amount present in 1 c.cm. of urine, and that this urine was diluted to 10 c.cm. with acid, of which only 6 c.cm. were used, single logarithm paper being used for the curve. As is well known, up to a certain concentration, the turbidity and light-

GRAPH I

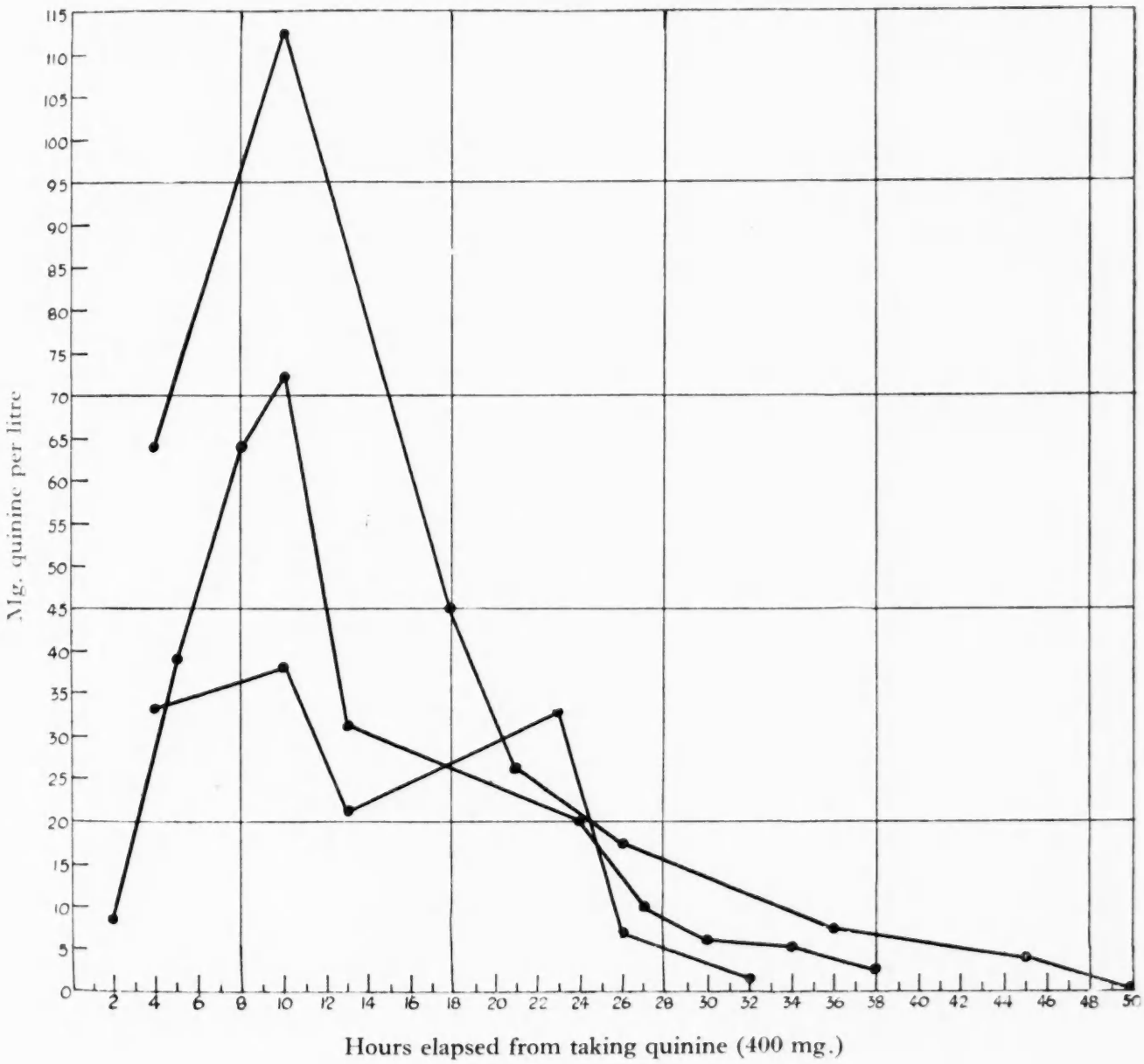
Concentration-curve showing effects of light-absorption in solutions of high turbidity



reflection are directly proportional ; but at higher concentrations the Tyndall effect is interfered with by light-absorption in consequence of the great turbidity of the fluid. A concentration-curve made up for solutions that pass from very dilute strengths to high concentrations will have the form shown in the curve in Graph 1. From this it will be obvious that it is impossible to decide on which side of the peak any reading must be plotted. To ascertain this all that is necessary is to add water to the unknown ; if this dilution results in an increase of brightness, then the readings must be taken as lying on the right-hand side of the peak of the curve ; if the dilution results in a decrease of brightness, the readings must be taken as on the left-hand side of the peak. The reason for this is that solutions whose readings fall on the right of the peak will have their reflection

GRAPH 2

Showing differences in urinary excretion of quinine by the same individual after a standard dose of 400 mg. (Constructed from a group of analyses.)



values decreased by light-absorption on account of their great turbidity, and as the dilution decreases their turbidity there will be a consequent fall in the quantity of light absorbed, thereby increasing the amount of light that is reflected. If a spectrophotometer with its nephelometric attachment is not available, then an ordinary nephelometer of the Kober type may be used. With such instruments standard solutions will be necessary, and, as already pointed out, these standards should be as near as possible to the value of the unknown. If the Kober type of nephelometer is used, the precautions already outlined must be carefully observed. The formula for calculating is as follows :—

$$\frac{S}{U} \times \text{concentration of standard} = \text{mg. quinine in amount of filtrate taken (= 6 c.cm. or 3 c.cm.)}$$

where S = Height of standard.
,, U = ,, unknown.

The 6 c.cm. of filtrate represent 6/10 of one c.cm. of urine (or whatever dilution is made), from which can be calculated the amount per litre or sample.

FAECES

Discussion

The quantitative estimation of quinine in faeces cannot be carried out with the same degree of precision as that for blood and urine, for the reason that the quinine is not distributed uniformly throughout the faecal mass. To some extent this can be overcome by taking samples from every part of the faeces, but such means cannot be regarded as entirely satisfactory. Alternatively, the stool may be passed into a tared pan, and the whole taken for analysis ; this is really the only satisfactory means, if it is desired to ascertain the ratio between renal and alimentary excretion.

Another rather serious limitation, unless steps are taken to overcome it, is that the ether extracts certain pigments from the food residues of vegetable origin. Two such pigments seem to be of importance—chlorophyll, and a diphenyl-methane derivative of skatole or some related pyrrol group. This latter substance has been described by Hewitt (1927), who states that a dried methyl alcohol solution of the pigment which he has prepared exhibits an absorption band at about 5,600–5,800 A°, the purified substance giving a faint band at 4,900–5,100 A°. This substance is present in normal faeces, and has been shown to be independent of dietary influences. Since its absorption is known it can be compensated for by the use of appropriate spectral filters. With regard to chlorophyll, this will only interfere with the analysis if much chlorophyll-containing food has been consumed immediately prior to, or during, the experiment, or if there is some catarrhal condition of the upper digestive tract (Cammidge, 1914), in both of which cases chlorophyll will appear in the faeces, and the acid that is added at the end of the reaction will form with chlorophyll-A an olive-green derivative, and with chlorophyll-B a red-brown one (Willstaetter

and Stoll, 1913). The chlorophyll can be identified by means of the spectroscope, and accounted for in the final analysis by the use of spectral filters. We have, however, discovered that all absorption can be eliminated from the final solution by the simple process of thoroughly alkalizing the faeces before the extraction is commenced (see below), and by this means a perfectly clear final solution is obtained, which is ideal for nephelometric work. This alkalization is convenient in cases where a spectrophotometer is not available, since it obviates the use of spectral filters for the elimination of the absorption bands. Alternatively, the colour may be adsorbed by means of specific adsorption. After a number of trials we found that Permutit (synthetic aluminium silicate, prepared by the Permutit Co. of New York) is effective in adsorbing the colour but leaves the quinine still in the solution. Black silica oxide, and barium carbonate may also be used, but we are uncertain as to their action on quinine.*

As already pointed out, we have not so far encountered a substance, other than the alkaloids mentioned, that gives a positive reaction with silico-tungstic acid ; but it is conceivable that, with the immense variety of food consumed in different parts of the world, one may be found to give a positive reaction.

In order to facilitate the extraction, it is well to give the patient a diet that leaves the least amount of residue and is as free as possible from vegetable pigments, although we have quite satisfactorily carried out many of our analyses on a full mixed diet. The amount of quinine excreted in the faeces passed during the 48 hours of the experiment is, like that for urine, subject to very wide variations, ranging from a minimum of 12 mg. to as much, in some cases, as 300 mg. in the whole stool, after a dose of 400 mg. of the sulphate. Here again we are uncertain to what these wide variations are due ; the solubility of the drug may have some effect, as well as the consumption of substances that facilitate absorption, and the intensity of peristalsis. An account of this aspect of the subject both as regards faeces and urine will be published later.

For our analyses we have taken either the whole stool or 5 or 10 gm. taken from every part of the faeces ; the former method is to be preferred, if accuracy is desired.

* It should be borne in mind that the amount of substance adsorbed is not in direct linear relation to the concentration of the adsorbed substance in the solution. Thus if A represents the amount of substance adsorbed from a given solution, then the amount adsorbed from a solution twice that strength will not be A $\times$ 2, but A $\times$ some root of 2 (i.e., less than two). In other words, the more dilute the solution the greater the quantity of its contents that is adsorbed. This process can be expressed by Freundlich's adsorption isotherm, which satisfies the adsorption process over a wide range, but breaks down at high concentrations. The equation is as follows :— $\frac{X}{M} = kc$; where M = the weight of adsorbent present ; k = a constant depending on the nature of the adsorbent and is the amount adsorbed when c = 1 and n = a constant depending on the nature of the adsorbed substance, and is usually 0·5. Expressed logarithmically the equation will be :—

$$\log \frac{X}{M} = \log k + \frac{1}{n} \log C.$$

Method

The faeces are passed, urine-free, into a tared pan, and their weight is estimated. Either the whole stool or an aliquot portion is turned into a large vessel (we have used a stoppered Erlenmeyer flask), alkalized with 5–7 c.cm. of concentrated ammonium hydroxide, and emulsified with enough distilled water to form a thin paste, which must be free from all lumps. To this watery paste ether is added so as to form a layer about 5 or 6 cm. above the paste. The whole is now very thoroughly shaken for 10 minutes, preferably on a mechanical shaker, and set aside for one hour, being occasionally shaken. The etherial mass is now centrifuged, and the upper ether layer removed and placed in a flask. More ether is added to the paste, shaken, centrifuged, and the second etherial layer removed and added to the first. This process of adding ether, shaking and centrifuging is repeated in all two or three times, according to the amount of faeces being dealt with. The collected etherial layers are now filtered, and the filter-paper is washed down with a few c.cm. of ether (the filtrate usually comes through greenish-yellow). This filtrate is evaporated to dryness in a water-bath, and, when quite dry, the residue is taken up in 5 or 10 c.cm. of 0·5N. HCl acid. (The amount of acid used to take up the residue will depend on the amount of quinine expected, and this can be gauged by taking into account the size of the dose of quinine given, the time that has elapsed and the number of stools passed ; rarely will more than 10 c.cm. of acid be needed.) The acid extract is returned to the water-bath for a few minutes to facilitate the solution of the quinine, and while still hot filtered through a Whatman 42 paper. The filtrate should come through crystal-clear and free of all colour ; should there be a reddish-brown tinge, too little ammonia was used for the initial alkalization ; this tinge can be adsorbed by means of Permutit, followed by filtering.* An aliquot portion of the filtrate is treated with silico-tungstic acid (0·06 c.cm. if 3 c.cm. of filtrate are taken, or 0·12 c.cm. of acid if 6 c.cm. of filtrate are taken). Place in the boiling water-bath for 5 minutes, cool rapidly under the tap, and read in the nephelometer as for urine, bearing in mind the amount of faeces taken and the acid dilution factor.

BLOOD

Discussion

The amount of quinine in blood ranges from a minimum of 1 mg. per litre to 10 mg. per litre. It is therefore sufficient for the analysis if 3–5 c.cm. are taken for extraction, though we have successfully used 1 c.cm. of blood. As already stated, the method of Ramsden and Lipkin was found to be altogether too cumbersome for general use ; nor is their method as modified by Acton and King very much better. The fluorescence method of Pantschenkow and Kirstner (1928), although accurate and simple, requires an ultra-violet radiation

* The filtrate is shaken with about 100 mg. of Permutit, and then filtered ; the clear filtrate is used for the analysis.

apparatus. The colorimetric method of Vedder and Masen was found by spectrophotometric analysis to be subject to such variations as to render it of doubtful value. It was therefore decided to modify the nephelometric method of these authors, and to use it for all our blood work.

In their account of blood quinine analysis, Vedder and Masen recommend that 5 c.cm. of blood should be pipetted on to a quantity of long fibre asbestos packed into a specially made tube with a constriction about its middle, and that the material should be extracted forthwith. In our opinion this is an unsatisfactory procedure, because it is difficult, or even impossible, to pipette such a large quantity of blood on to asbestos in a tube, without a considerable amount of the blood seeping through the asbestos and adhering to the walls of the glass container. Further, if the asbestos is first packed into the tube and the blood delivered on to it in the tube, it will be found that the asbestos nearest the top becomes soaked with blood, with consequent oozing through on to the glass, whilst the asbestos at the bottom of the tube absorbs no blood, and is for all practical purposes useless. It is therefore better, if asbestos fibre is to be used, to put it into an evaporating basin spread in a layer at the bottom of the basin and to pipette the blood on to it here ; thus a uniform distribution of the blood can be secured. The asbestos and blood should not be now extracted, as Vedder suggests, but should be placed in a dessicator and allowed to dry overnight.

When dry, it can be placed in the extraction-vessel. If this preliminary drying is omitted, not only will the blood and asbestos stick to the walls of the extraction-vessel, but also the ether will carry down pigments, etc., from the wet mass, cloud the final product and vitiate the nephelometric estimation. If the preliminary drying is carried out, however, none of these things will happen, and the special tube recommended by Vedder will not be necessary, since it was designed to prevent the blood from running down the inside of the tube, and an ordinary test-tube with holes drilled at the bottom can be used to put the blood-asbestos-mixture into.

The asbestos used must not be acid in reaction, as this will interfere with the quantitative recovery of quinine. We have used acid- and alkali-washed asbestos, the base being used last, as specified by Prengl for micro-analysis ; ordinary crude asbestos is sometimes liable to give variable results. The use of the salt-bath for evaporating the ether and drying the residue seems to be fraught with some danger, as salt particles are difficult to exclude, and should they gain entrance may seriously affect the result.* With urine and faeces an ordinary water-bath is sufficient ; but frequently with blood there remains after evaporation a few droplets in the tube, that will not evaporate with the temperature of

* It is possible that the reason for this is that the precipitation of the alkaloid is due to the adsorption of the complex negative ions by the positively charged colloidal particles, and it is suggested that the precipitating substance can only be effective in acid solution, in which the proteins will carry a positive charge, so that the entrance of salt particles by altering the pH will seriously upset the reaction.

the boiling water-bath, and we have used an oil-bath to dry up this residue. If a salt-bath is used, then some precautions must be taken to see that no salt particles enter the inside of the tube. Roy (1926), using Tanret's reagent after the method of Ramsden and Lipkin, and noting the danger of salt particles entering the tubes, has condemned its use, claiming that with Tanret's reagent it is unnecessary.

In most of our work we have used asbestos powder, and have carried out the extraction in the ordinary filter-paper thimbles, such as are used in the Myers and Wardell blood cholesterol method. Our feeling is that this is not only more convenient than fibre, but permits more complete extraction on account of the more even mixture of blood and asbestos, no thick masses forming and matting together, and the final state of the powder and blood being a fine uniform suspension through which the extraction fluid can percolate with ease. If this asbestos powder is used, a sufficient quantity to absorb the amount of blood that is going to be used should be placed in an evaporating basin, a ' well ' scooped into it, and the blood placed into this well, care being taken to see that no blood comes into contact with the walls of the basin. Asbestos powder is then placed on top of the blood, and the basin put in the desiccator for 24 hours. The blood and asbestos must not be stirred immediately after the pipetting of the blood on to the powder, because they will not ' mix ' ; the asbestos will, however, slowly absorb the blood, and when this has happened the two can be mixed with ease, broken up, and placed in the extraction apparatus.

Alternatively, $NaHCO_3$ powder may be used as in the fluorescence method, and extracted in the same way as asbestos powder.

In packing the asbestos powder or fibre into their respective extraction-cups, care should be taken to see that it is packed in such a way that the ether will not run rapidly through the mixture, but will form a shallow layer on the top of the asbestos-blood-mixture, whereby the whole of the mixture must be percolated by the extracting fluid, and not merely by the spot where the ether drops (Foy and Kondi, 1935).

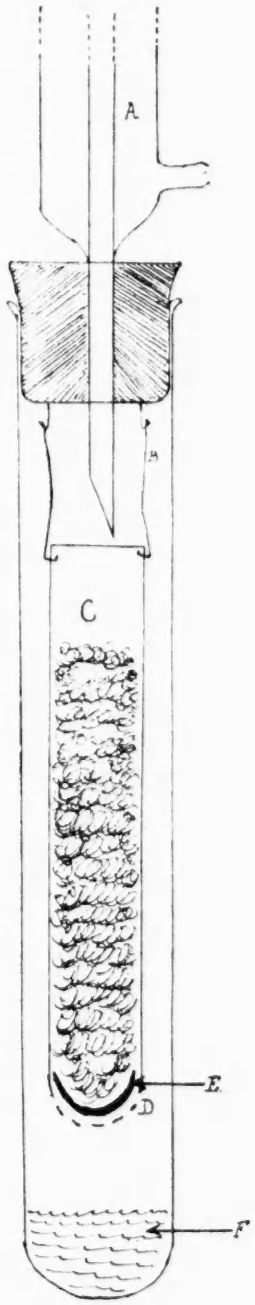
Method

Pipette 1–5 c.cm. of whole blood, serum, plasma or cells on to either asbestos fibre or powder, as described above, and allow to dry in a desiccator overnight or longer. When thoroughly dry, transfer to the appropriate extraction-vessel—either a filter-paper thimble in a glass shell, as in the Myers and Wardell blood cholesterol method, if asbestos powder is used ; or a test-tube with holes drilled at the bottom, if fibre is used. (To prevent any of the fibre from falling through the holes a piece of filter-paper is cut and placed at the bottom, over the holes.) The vessel containing the blood and asbestos mixture is suspended in an outer tube attached to a condenser, as shown in diagrams A and B *infra*. About 10 c.cm. of ether are now placed in the outer tube and the apparatus is set up on a water-bath and extracted for from 3–5 hours. During the extraction,

see that the shallow layer of ether remains above the asbestos, as already mentioned.

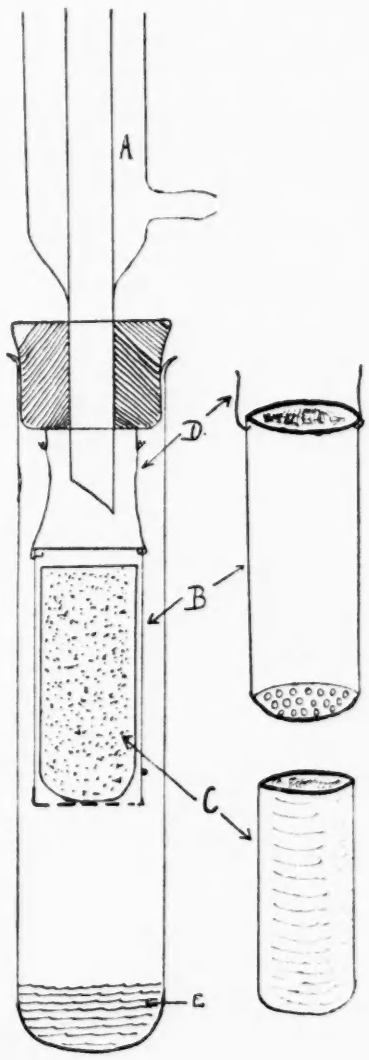
When the extraction is complete, dismantle the apparatus, wash the asbestos containers with a little ether. Evaporate the ether to complete dryness on a boiling bath for about 10 or 15 minutes. When all is quite dry within the vessel, add 5 c.cm. of 0·5N. HCl acid, and return to the bath for a few minutes to facilitate

DIAGRAM A



Apparatus for use with asbestos fibre. A.—Condenser ; B.—Hooks ; C.—Glass tube containing fibre ; D.—Holes in glass tube ; E.—Circlet of thick filter-paper ; F.—Ether.

DIAGRAM B



Apparatus for use with asbestos powder or $NaHCO_3$. A.—Condenser ; B.—Glass container with holes at bottom, for thimble ; C.—Filter-paper thimble ; D.—Hooks ; E.—Ether.

the solution of the quinine. Filter through a Whatman 42 paper. The filtrate should be crystal-clear.

Take 3 c.cm. of the filtrate, add 0·06 c.cm. of 10 per cent. silico-tungstic acid, return to the boiling bath for 5 minutes. Cool rapidly and read in the nephelometer as for urine and faeces. In making the calculation or the concentration-curve, it is to be borne in mind that the 5 c.cm. of acid added takes up all the quinine that was in the amount of blood taken (3 or 5 c.cm.), and that the amount of filtrate taken represents 3/5 of the amount of blood taken ; that is, either 3/5 of 3 c.cm. or 3/5 of 5 c.cm. of blood. From these figures the amount of quinine per cent. is calculated.

SUMMARY

1. A new photo-nephelometric method is described for the quantitative estimation of minute amounts of quinine in faeces and body fluids, which is accurate and sensitive to 0·5 mg. per litre.

2. The use of a new photo-nephelometer, for use with a standardized turbidity prism, is briefly described.

3. Variations in the amount of quinine excreted in the urine after similar doses are noted.

4. Large quantities of quinine were found to be frequently excreted in the faeces.

5. The discrepancy between the amount of quinine absorbed, as shown by the urine level and blood quinine level, is noted.

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THE BRITISH SPECIES OF THE SUBFAMILY SARCOPHAGINAE, WITH ILLUSTRATIONS OF THE MALE AND FEMALE TERMINALIA

(*Continued from page* 90)

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INTRODUCTION

In this paper, which forms the second of this series, we shall describe the adults of four more species of *Sarcophaga* and illustrate their terminalia. For want of space and time, we have decided to omit drawings of the entire ♂ terminalia as seen in side view, and to limit the notes on the terminalia only to the important diagnostic characters of these structures, comparing them with those of others. The study of the ♀ terminalia of these additional species has forced us to the conclusion that the plate attached to the distal end of sternum 7 is most probably sternum 9. In the ♀♀ of this group of flies we have repeatedly noted that there is a tendency for the distal sternal plates to become fused with each other, and this seems to be especially the case with sternum 7 and sternum 9.

Sarcophaga pumila Mg. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN TERMINALIA. ♂. *S. pumila* is one of the smallest of our species : we have specimens less than 4 mm. in length over-all ; but larger individuals do occur, and our largest is 9 mm. long. It belongs to the group of species with black terminalia, 3 d.c., and no marginal setae on the third abdominal tergum. Having no setae on the first longitudinal wing vein, it can readily be separated from *dissimilis* Mg., with which it might be confused on account of similar size, habits and general appearance ; moreover, it has no presutural acrostichals. Apart from its smallness it should be fairly easily recognized by its very slender terminal segments, which are longer in proportion to their thickness than in other British species, the first (abdominal tergum 7 of the figures) being quite twice as long as it is wide. It can also be separated from the species which run down near to it in the keys, *melanura* Mg., *incisilobata* Pand. and *hirticrus* Pand., by the total failure of the apical scutellar setae, the discal pair being placed unusually far back so as almost to take the place of the apicals ; also, the prescutellar acrostichals are absent or very small. *Melanura* is a large, very different-looking species, with wider frons, which is four-fifths of the width of a single eye ; *hirticrus* is also larger and different-looking, is unusually densely pilose on all

the terminalia, and lacks the costal thorn, which is present in *pumila*; and *incisilobata*, some individuals of which are similar in size and general appearance to large *pumila*, has a patch of grey tomentum on tergum 7, whilst that of *pumila* is entirely shining; moreover, it has no marginal setae on the hinder edge, whilst *pumila* has a row of strong ones.

Pumila has frons three-fifths of eye-width; short antennae, the third joint barely half as long again as the second; genal setae few and only moderately strong; no presutural intra-alar; long costal thorn; third costal section only a trifle longer than the fifth; lamellae of apical sternum armed with brush-like mass of short setae. LEG CHAETOTAXY. Hind tibiae with fringes of long hairs, especially postero-ventrally; mid tibiae with similar long hairs, but fewer; on the femora the usual ventral series of setae very slightly developed, scarcely forming any comb-like row even on the apical portion of the mid femora. The terminal segments sometimes show some reddish colour, chiefly dorsally on tergum 7.

♀. In this sex *pumila* runs down in the keys to the same group of species as the ♂, and can be recognized by much the same characters. It is distinguished from *melanura* by the much narrower frons, which is about eye-width, apart from size and general appearance; from *hirticrus* by its long costal thorn, again apart from size and facies; and from *incisilobata* by the absence of prescutellar acrostichals, and by the scutellum, on which not only do the apicals fail (which is the case in this sex of most of the species), but the discals also are nearly or quite wanting, leaving just two strong bristles on each side. In most other respects the ♀ agrees with the ♂, excepting for the usual sexual differences; there are no fringes of hairs on the tibiae, and the usual ventral rows of setae are even more irregular and ill-developed than in the ♂.

NOTES. *S. pumila* is a fairly common and widely distributed species in this country. It seems specially to favour chalky downlands, but we have it from a variety of other localities, Chippenham Fen, Studland, Bagley Wood, West Malvern, etc., etc. Abroad, it has been recorded from Germany, Austria, Hungary, Italy and France. Lundbeck says that it is rare in Denmark, but has occurred in ' all Europe to Middle Sweden.' When dissecting the abdomen of a ♀ we have noted only a relatively few large first stage larvae.

DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. The ♂ terminalia of *pumila* are illustrated in fig. 1; they should be compared with those of *dissimilis* illustrated in our first paper. Sternum 5 of *pumila* (fig. 1, *j*) is smaller and narrower (particularly the posterior processes) than that of *dissimilis* (fig. 1, *g*) drawn to the same magnification. The anal cerci (fig. 1, *d*) of *pumila* are shorter, and the ends are more pointed and bent inwards than those of *dissimilis*. The phallosome of *pumila* (fig. 1, *a*) is very similar to that of *dissimilis*, but has two distal dorsal processes, whereas that of *dissimilis* has only one. The posterior part of the paramere of *pumila* (fig. 1, *a*, *e*, *h*) is very like that of *dissimilis* and is hooked at the end; the anterior paramere of *pumila* (fig. 1, *a*, *e*, *h*) is narrower than that

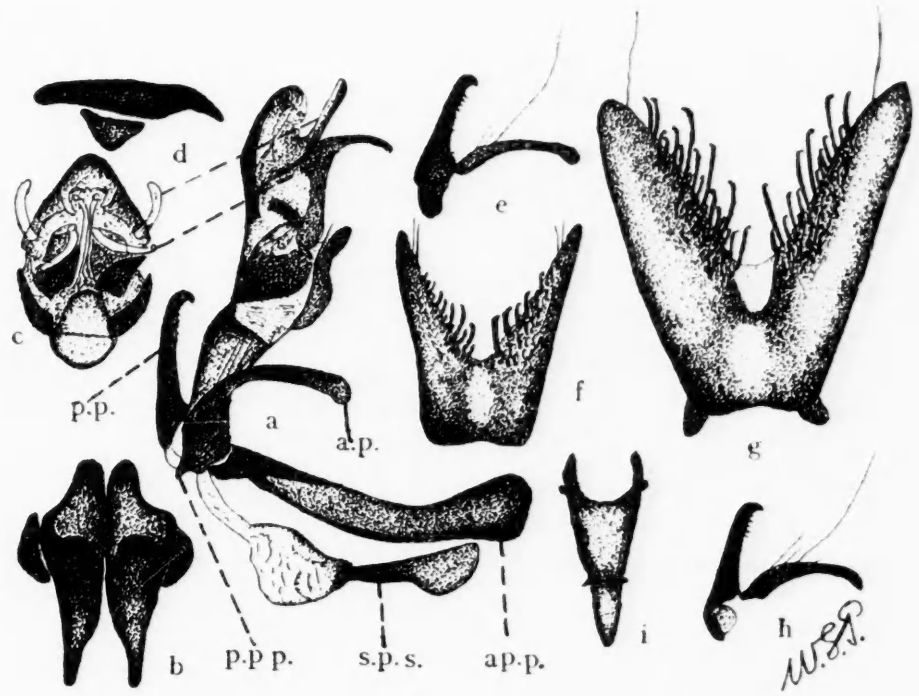

FIG. 1. *a.*—Phallosome and one paramere of *pumila* in side view ; *a.p.*—Anterior part of paramere ; *ap.p.*—Apodeme of phallosome ; *p.p.*—Posterior part of paramere ; *p.p.p.*—Posterior process of phallosome ; *s.p.s.*—Sperm pump sclerite ; *b.*—Ventral view of anal cerci and distal segments of ninth coxites ; *c.*—Dorsal view of end of phallosome ; *d.*—Lateral view of anal cercus and distal segment of ninth coxite ; *e.*—Lateral view of right paramere ; *f.*—Fifth sternum ; *g.*—Fifth sternum of *dissimilis* (same scale for comparison) ; *h.*—Lateral view of left paramere ; *i.*—Ninth tergo-sternum (British specimen).

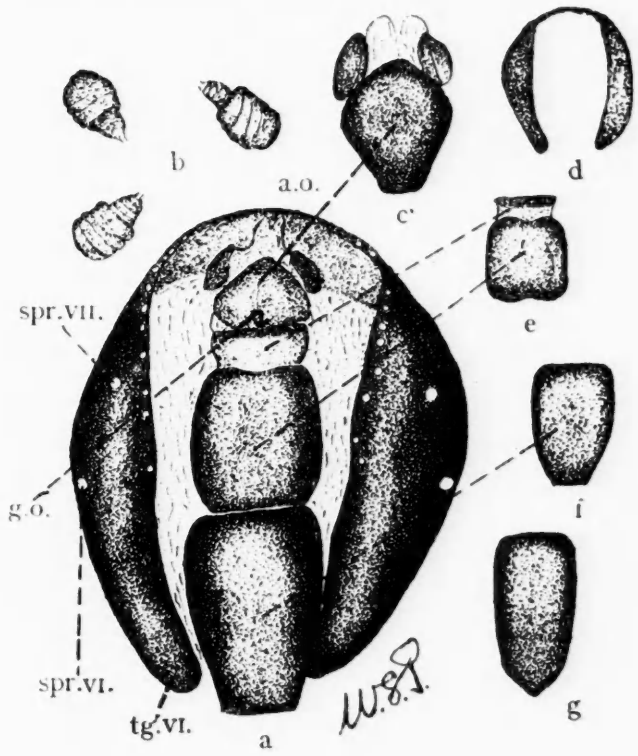

FIG. 2. *a.*—Ventral view of terminalia of ♀ *pumila* to show diagnostic characters ; *a.o.*—Anal opening ; *g.o.*—Genital opening ; *spr. vi*, *spr. vii.*—Sixth, seventh spiracle ; *tg. vi.*—Sixth tergum ; *b.*—Spermathecae ; *c.*—Tenth sternum and anal cerci ; *d.*—Sixth tergum showing that it consists of a single plate ; *e.*—Seventh, ninth (?) terga ; *f.*—Sixth sternum ; *g.*—Fifth sternum (British specimen).

of *dissimilis*, which is more pointed and bent and is armed on its curved ventral surface with a more continuous row of hairs ; that of *pumila* usually has one very long hair in addition to two or three smaller ones.

♀. The ♀ terminalia of *pumila* are illustrated in fig. 2. It is interesting to note that in *pumila* as well as in *dissimilis* tergum 6 is a single plate. These (as well as *falculata* and *haemorrhoa*) are the only ♀♀ of the British species in which we have so far noted this character. The plate (sternum 9) at the end of sternum 7 in *pumila* (fig. 2, *e*) is better developed than in *dissimilis*. As already noted, we believe that this plate is most probably sternum 9 which has now become fused with sternum 7. In *dissimilis*, sternum 10 has attached to it a very distinct and somewhat triangular plate, which appears to form the posterior wall of the genital opening ; in the drawing of the terminalia of *dissimilis* it was described as sternum 9. We are now very doubtful as to its true identity, for it may only be an extension of sternum 10. It can readily be seen by hooking back the genital opening. There is no such plate in *pumila* (fig. 2, *a*). In both species there is no signum or chitinous plate to the uterus ; and terga 7, 9 and 10 are wanting in both.

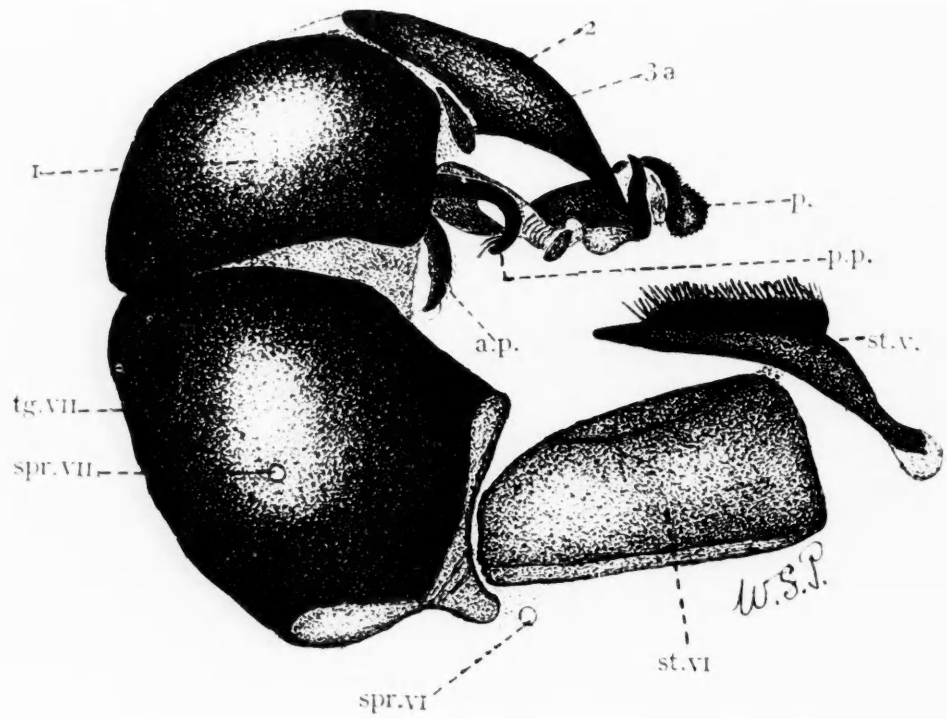

FIG. 3.—Terminalia of ♂ *rosellei* in side view. *st. v, st. vi.*—Fifth, sixth sterna ; *spr. vi, spr. vii.*—Sixth, seventh spiracle ; *tg. vi, tg. vii.*—Sixth, seventh terga ; 1.—Tenth tergum ; 2.—Anal cercus ; 3*a*.—Distal segment of ninth coxite ; *a.p.*—Anterior paramere ; *p.*—Phallosome ; *p.p.*—Posterior paramere (British specimen).

Sarcophaga rosellei Böttcher. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN TERMINALIA. ♂. In the keys, this species runs to the group with black terminalia, no setae on first longitudinal vein, no presutural acrs., 3 d.c., and possessing marginals on second visible tergum ; it has to be compared with

crassimargo Pand., *agnata* Rond., *filia* Rond. and *pumila* Mg., with the same combination of characters. It is a difficult group, and without examination of the terminalia no confidence can be felt in any identifications ; but there seem to be some external characters which may be helpful. Already, when describing *crassimargo* and *pumila*, we have dealt with such distinctions as we have been able to find between those two species and *rosellei*. *Agnata* Mg. is the species most closely related to *rosellei*, and we have even been in doubt about some specimens after examining their terminalia. *Agnata* seems to be variable, and, although typical specimens are distinct enough, others vary in the direction of *rosellei* : even the phallosome varies and must be closely observed for certainty. Normally, the frons of *agnata* is the narrowest in this group, being barely two-fifths of the width of an eye ; but it is sometimes wider. Perhaps the most reliable character is the presence of some presutural macrochaetae, irregular and not very strong, but, we think, always present in *agnata* and never in *rosellei* ; further, *agnata* has long pilosity on all the sterna, whilst in *rosellei* there is some, less developed, on the second sternum only. *Filia* Rond., which runs down to

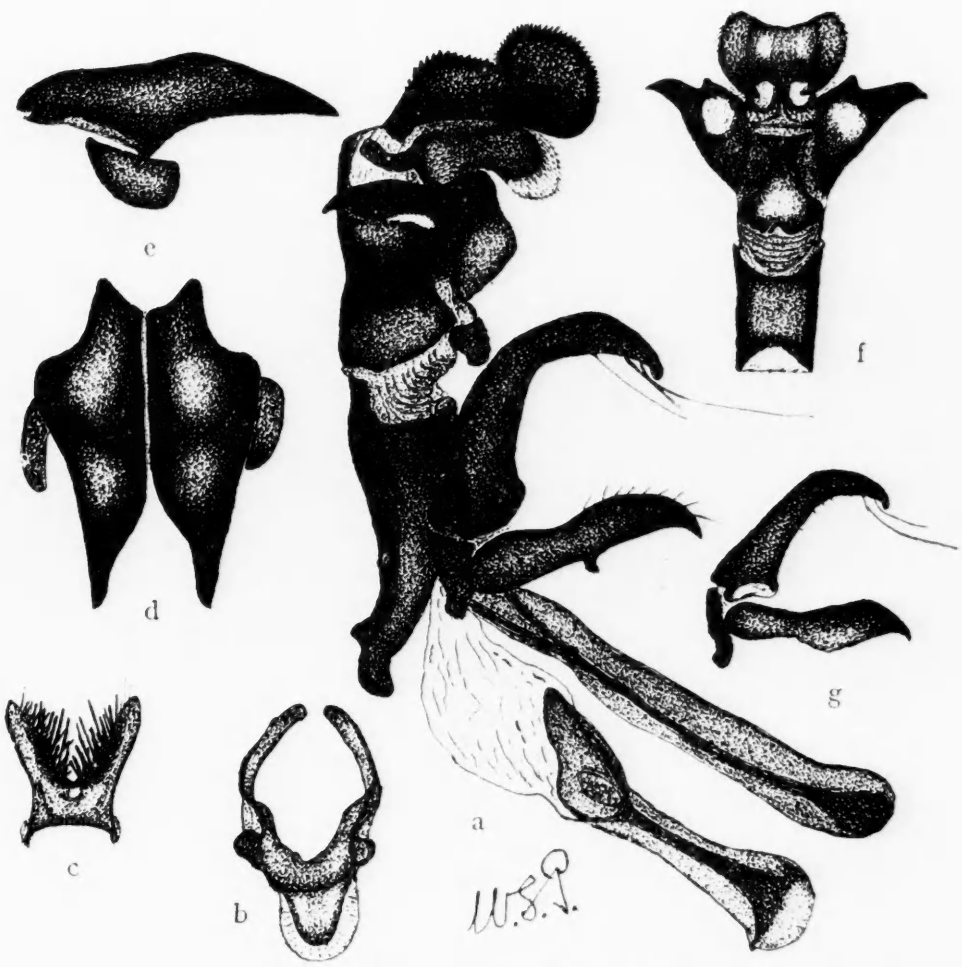

FIG. 4. *a.*—Phallosome and one paramere of *rosellei* in side view ; *b.*—Ninth tergo-sternum ; *c.*—Fifth sternum ; *d.*—Ventral view of anal cerci and distal segments of ninth coxites ; *e.*—Lateral view of anal cercus and distal segment of ninth coxite ; *f.*—Dorsal view of end of phallosome ; *g.*—Lateral view of right paramere (British specimen).

the same group in keys, is a different-looking fly, larger, browner, and with a decidedly wider frons, which is fully four-fifths the width of an eye, that of *rosellei* being about three-fifths. *Sinuata* Mg., which otherwise might come into this group, can be recognized at once by the patch of golden pubescence at the tip of the middle femora. *Incisilobata* Pand. likewise is closely related, but in this sex should be distinguished by the failure of marginals on its second tergum.

S. rosellei is a medium-sized species, about 8–10 mm. long, blackish in general tone, with frons about three-fifths of eye-width ; frontalia about twice as wide as an orbit at its narrowest point ; antennae rather large, third joint about twice length of second ; genal setae strongly developed ; strong prescutellar acrs.; scutellum with well-developed apicals and discals ; tergum 7 with some silvery tomentum, and a row of not very strong hind marginal setae ; sternum 5 with well-developed brush-like masses of setae on the lamellae ; a short costal thorn ; third costal section nearly as long as the fifth and sixth together. LEG CHAETOTAXY. Hind tibiae with long fringes of hairs on antero-

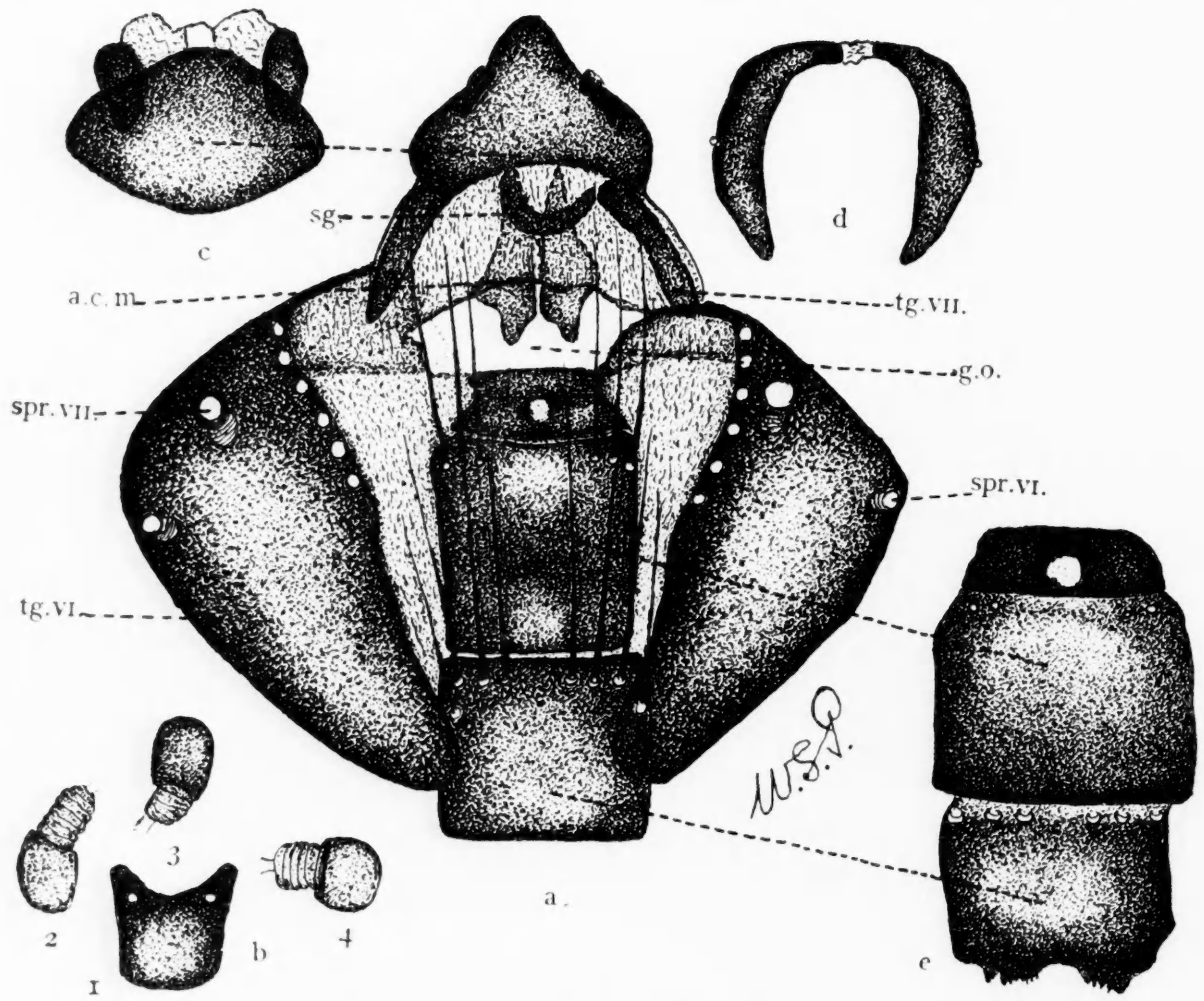

FIG. 5. *a.*—Ventral view of terminalia of ♀ *rosellei* to show diagnostic characters ; *a.c.m.*—Anal cerci of ♂ hooked into genital opening; *tg. vi, tg. vii.*—Sixth, seventh tergum; *sg.*—Signum (chitinous plate of uterus) drawn back by anal cerci of ♂ ; other lettering as in fig. 2, *a* ; *b.*—1, Signum ; 2, 3, 4, Spermathecae ; *c.*—Tenth sternum and anal cerci ; *d.*—Sixth tergum to show its division into two plates ; *e.*—Sixth, seventh, ninth (?) sterna (British specimen).

and postero-ventral aspects ; mid tibiae also pilose, but no long fringe-like series ; mid femora with antero-ventral row irregular, with short scattered bristles on basal portion, becoming a little finer and more regular towards apex ; postero-ventral series complete, nearly all long fine hair-like bristles, with no comb-like row at apex ; hind femora with antero-ventral irregular but nearly complete series of long fine hair-like bristles ; postero-ventral series little developed, some long hairs towards apex.

♀. Fortunately we had three pairs of this species taken *in cop.*, so that there is no doubt about this sex. But, in spite of having determined ♀♀, it has proved almost impossible to find reliable external characters by which it could be separated from its allies. *Sinuata* Mg. ♀, like the ♂, has the patch of golden hairs at the tip of the mid femora ; *melanura* Mg. ♀ has a much wider frons—nearly half as wide again as an eye—and is larger and browner in colour ; *pumila* Mg. has two pairs of bristles on the scutellum, the discals failing, and is usually smaller ; but there remain *agnata* Rond., *crassimargo* Pand., *incisilobata* Pand., *filia* Rond. and *rosellei* Bött., which are so much alike that on external characters we cannot separate them with any confidence. *Agnata* has the narrowest frons, and can probably be recognized by the long presutural acrostichal bristle-like hairs ; *filia* has perhaps a comparatively narrower frontalia in proportion to width of frons, being little wider than a single orbit ; *incisilobata* belongs to those species without marginals on the second tergum, but this character is unreliable in the female sex as they are never so well developed even in those species the ♂♂ of which usually have them ; *crassimargo* might be identified by the wide flat edge to the first abdominal tergum, but that again is a character of not much value in this sex, as this segment tends to be flattened in the ♀♀ of most of the species.

S. rosellei has frons of eye width ; frontalia about half as wide again as an orbit ; scutellum with 3 pairs of marginal setae and no apparent discals ; all the bristle-series on the mid and hind femora are weak and irregular ; other characters as in ♂.

Notes. *S. rosellei* is a comparatively rare species. F. Jenkinson took a series at Cambridge about 1904, chiefly in his garden. Mr. A. H. Hamm and Mr. J. Collins have taken a fair number in and around Oxford, and we have taken or examined specimens from Princes Risborough, Clayton in Sussex, Tunbridge Wells (Col. C. G. Nurse), Plymouth (Col. Nurse), Dorset (Dr. C. D. Day), etc. It can be got in fair numbers in Burton Pynsent Woods, Curry Rivel, Somerset, in August and September. Böttcher described it from a Wiesbaden specimen, and records it from two or three other German localities and from Upper Austria. Lundbeck does not include it in his Danish list. Hence it seems to be rare on the continent.

Diagnostic Characters of Terminalia. Here we should like to correct an error which unfortunately crept into our first paper (see p. 73 *supra*) and for which one of the writers (W. S. P.) holds himself entirely responsible. Two

pairs fixed *in cop.* were provisionally identified as *crassimargo*, and their terminalia dissected and illustrated ; for some unaccountable reason the terminalia of ♂♂ correctly identified as *crassimargo* by the other author (C. J. W.) were not compared to check the provisional determination. The two pairs proved to be *rosellei*, but in the meantime the drawings of their terminalia had been wrongly published and described in our first paper as those of *crassimargo*. We reproduce the drawings again here, as well as those of the ♂ terminalia of true *crassimargo* for comparison. We have also illustrated what we believe to be the terminalia of the true ♀ of *crassimargo* ; but we can only offer them as representing that species with great diffidence and doubt, owing to the absence of pairs and to the difficulty of distinguishing it from its allies. We shall be very grateful to anyone who will supply us with a pair of *crassimargo* taken *in cop.*, to enable us to check our determination. It is not necessary to give any formal description of the terminalia of *crassimargo*, as a comparison with those of *rosellei* will suffice, the drawings speaking for themselves. The external characters of *crassimargo* were described in the first paper.

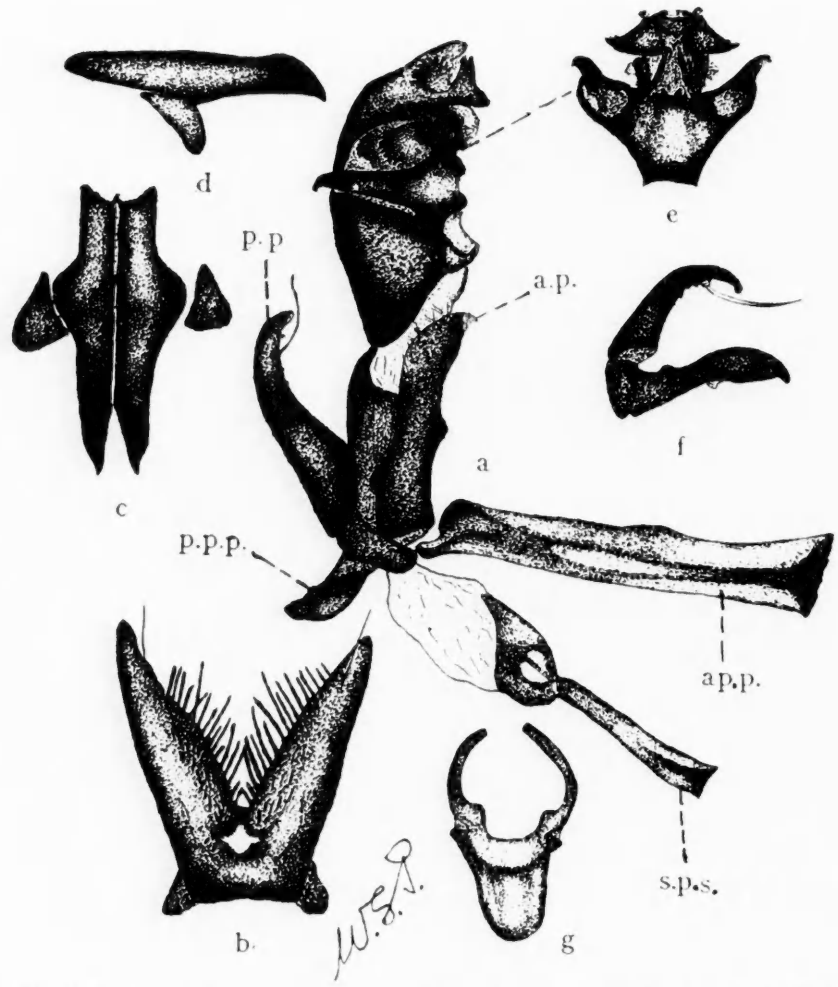

FIG. 6. *a.*—Phallosome and one paramere of *crassimargo* in side view ; lettering as in fig. 1, *a* ; *b.*—Fifth sternum ; *c.*—Ventral view of anal cerci and distal segments of ninth coxites ; *d.*—Lateral view of anal cercus and distal segment of ninth coxite ; *e.*—Dorsal view of end of phallosome ; *f.*—Lateral view of right paramere ; *g.*—Ninth tergo-sternum (British specimen).

Comparison between the Terminalia of *Rosellei* and *Crassimargo*. ♂. The ♂ terminalia of *rosellei* are illustrated in figs. 3, 4. They (especially fig. 4) should be compared with those of the terminalia of ♂ *crassimargo* (fig. 6), which they closely resemble. The most striking differences are as follows :—The anal cerci of *rosellei* (fig. 4, *d*, *e*) are straight, more pointed, longer and stouter than those of *crassimargo* (fig. 6, *c*, *d*) which are shorter and have broad and beak-like ends, especially when seen in side view. The phallosome of both (cf. figs. 4, *a* ; 6, *a*) has a pair of stout, ventrally-directed, horn-like processes, which are wider in *rosellei* than in *crassimargo* ; the distal end of the phallosome of *rosellei* (fig. 4, *a*) has a characteristic, rounded, very spiny plate, with a smaller proximal one ; in *crassimargo* there is an indefinite process at the distal end (fig. 6, *a*). In both the parameres are very similar, the posterior of *rosellei* being stouter than that of *crassimargo*.

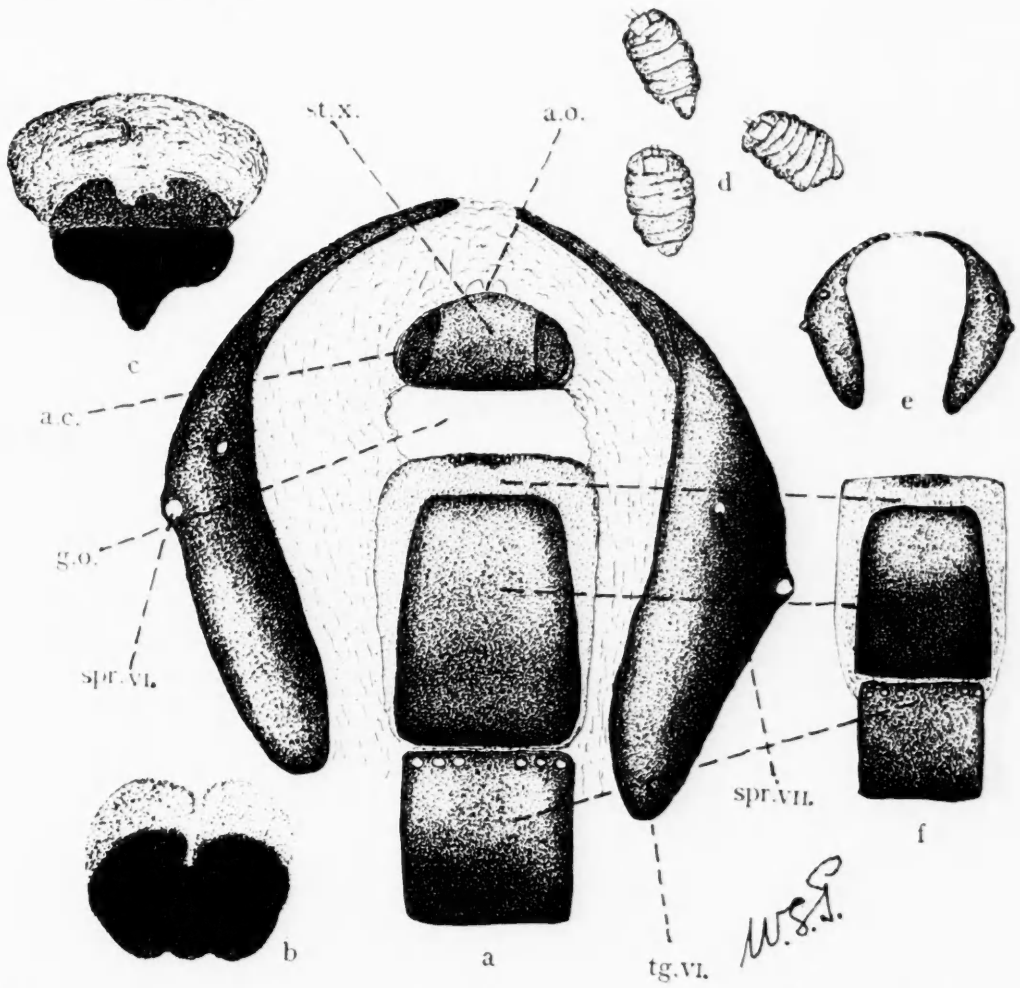

Fig. 7. *a*.—Ventral view of terminalia of ♀ *crassimargo* to show diagnostic characters ; *a.c.*—Anal cerci ; other lettering as in fig. 2, *a* ; *b*.—Ventral view of signum (chitinous plate of uterus) ; *c*.—Dorsal view of same ; *d*.—Spermathecae ; *e*.—Sixth tergum to show its division into two plates ; *f*.—Sixth, seventh, ninth (?) sterna (British specimen).

♀. The ♀ terminalia of *rosellei* are illustrated in fig. 5 ; it should be noted that they are in the position when seen *in cop*., sternum 10 being drawn back by the ♂ anal cerci, which are hooked into the genital opening. The ♀ terminalia

of what we believe to be those of *crassimargo* are illustrated in fig. 7. Though resembling each other, they are abundantly distinct and can readily be separated. In both, tergum 6 consists of two plates, and sterna 6 and 7 are similar, but the plate at the end of sternum 7 (sternum 9) is well developed in *rosellei*, and in the two ♀♀ examined had a round, light (clear) spot in the centre. In *crassimargo* this plate is very indefinite, lightly chitinized, and appears to surround sternum 7. Tergum 7 is present in *rosellei*, consisting of two narrow plates ; it is absent in *crassimargo*. Terga 9 and 10 are absent in both. The signum is well developed but strikingly different in the two species (cf. figs. 5, *b* ; 7, *b*, *c*).

Sarcophaga nigriventris Meigen. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN TERMINALIA. ♂. *S. nigriventris* is one of the smaller species. As it has black terminalia, bare first long vein, 3 d.c. and hind marginal setae on the second visible abdominal tergum, as well as some fairly well-developed presutural acrostichals, it need only be compared with *clathrata* Mg., *villeneuvei* Böttch., and perhaps with *agnata* Rond. *Clathrata* and *agnata* should be easily distinguished by their much narrower frons, which in *nigriventris* is nearly of eye-width, in *clathrata* little more than half eye-width, and in *agnata* still less. *Villeneuvei*, however, is similar in width of frons to *nigriventris*, and is so like in all respects that it is necessary to examine the terminalia for certain identification. Perhaps, however, the scutellum affords a distinctive character ; normally *nigriventris* has well-developed apical setae, which seem to fail entirely in *villeneuvei* ; the latter has the apex itself rather flattened.

S. nigriventris has frons of eye-width ; antennae with third joint about three-quarters as long again as second ; arista with short ciliation ; genal setae long and strong, but not numerous ; no presutural intra-alar ; third costal section short, a little longer than fifth ; long costal thorn ; sterna densely pilose, with many long hairs ; lamellae of apical (fifth) sternum with no brush-like masses of setae. LEG CHAETOTAXY. Tibiae without fringes; front femora with complete postero-ventral series of long bristles ; no antero-ventral series ; mid femora with postero-ventral series consisting of nearly equal, long bristles for whole length, with no comb-like row of shorter ones ; antero-ventral series similar, but becoming shorter and closer in apical third ; hind femora with antero-ventral row complete but rather sparse, and postero-ventral incomplete and irregular.

♀. This sex runs in keys to the same group of species with which to be compared. *Clathrata* again has a narrower frons, barely of eye-width, whilst in *nigriventris* it is considerably more than eye-width ; and, since *clathrata* is a dark blue-black fly, whilst *nigriventris* is yellowish-brown in general tone, these two can be readily separated. We have no ♀ *agnata*, but that species, like *clathrata*, would be distinguished by a narrower frons (certainly under eye-width), by the similar blue-black colour, and by the absence of costal thorn. *Villeneuvei*, however, of which again we possess no ♀, may be almost indistinguishable in that sex, as the apical scutellar character fails in this sex, these setae being absent alike in the ♀ of practically all species.

Nigriventris has a rather stout appearance, the abdomen being wide and thick ; it has the general characters given for the ♂, with exceptions already noted. It has a few long bristly hairs on the sterna, but none of the pilosity characteristic of the ♂.

NOTES. *Nigriventris* is not an abundant species, but we have specimens or records from a number of places—of which the majority seem to be at the seaside—from which it appears that it favours sandhills and similar spots. It has, however, also occurred in a variety of inland localities (Farley Mount, Hampshire, Chippenham Fen, Wyre Forest, etc.), and seems widely distributed over at least the southern half of England. On the Continent it is also widely distributed : Böttcher says in Germany, Hungary, France, Italy, Corsica and Spain, and Lundbeck includes it in his Denmark list, but says that it does not occur further north.

Böttcher records (teste Villeneuve) that it has been bred in France by Giard from *Helix cantianiformis* ; but one always suspects in the case of such records of *Sarcophaga* species that the host was merely carrion to the fly, and that there was no real parasitism or specific association. F. Jenkinson, however, had attached a note to one of his specimens that ' it seemed very interested in a snail shell.' Dr. K. G. Blair has a specimen with a note that it had been bred from *Blaps* (*mucronata* Latr.) ; but he tells us that he is unable to say whether it was bred from a dead or from a living beetle.

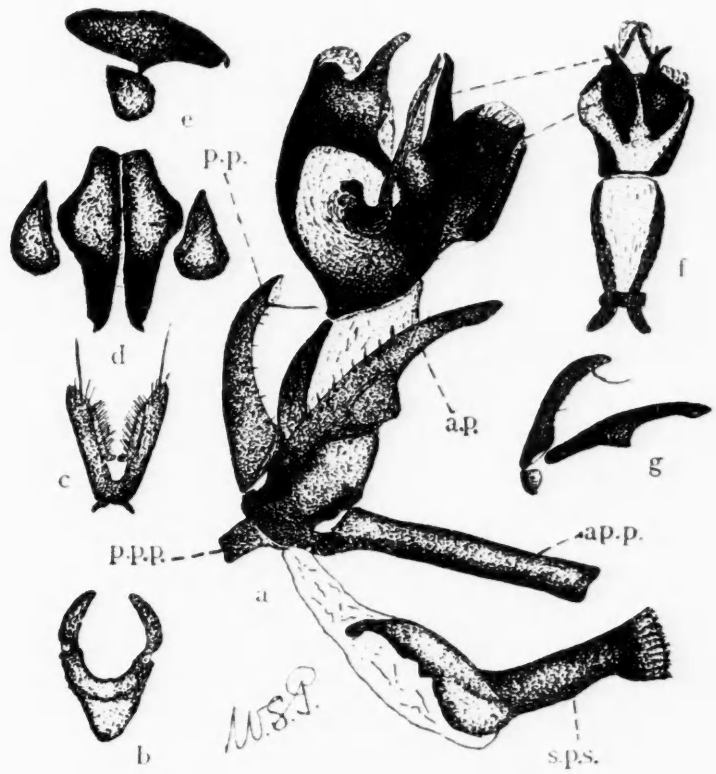

FIG. 8. *a.*—Phallosome and one paramere of *nigriventris* in side view ; lettering as in fig. 1, *a* ; *b.*—Ninth tergo-sternum ; *c.*—Fifth sternum ; *d.*—Ventral view of anal cerci and distal segments of ninth coxites ; *e.*—Lateral view of anal cercus and distal segment of ninth coxite ; *f.*—Dorsal view of phallosome ; *g.*—Lateral view of right paramere (British specimen).

Diagnostic Characters of Terminalia. ♂. The ♂ terminalia of *nigriventris* are illustrated in fig. 8 ; they should be compared with those of *clathrata* (illustrated in our first paper), which they closely resemble. The anal cerci of both are short, the distal end having a notch before the pointed end, which is longer in *clathrata* ; the anal cerci of *nigriventris* are distinctly shorter and wider than those of *clathrata* when seen in side view. The phallosome in both is short, the distal end broad ; in *nigriventris* (fig. 8, *a*) there is one stout, rod-like, chitinous process and two slenderer ones, whereas in *clathrata* there is only one stout, blunt rod. It is necessary to examine the phallosome very carefully with a fine needle in order to detect the slenderer processes in *nigriventris*. Projecting from the dorsal surface of the distal end of the phallosome of *nigriventris* there is a long, broad, flap-like process ; in *clathrata* there is in the similar position a rounded spiny plate. In *nigriventris*, at the distal end on the ventral side, there is a long pointed process, which is wanting in *clathrata*. The paramere is distinct in the two species, especially the anterior part, which is longer and wider in *clathrata* ; the posterior paramere in this species ends in a hooked point, depending on the angle at which it is seen ; in *nigriventris* the posterior part ends bluntly.

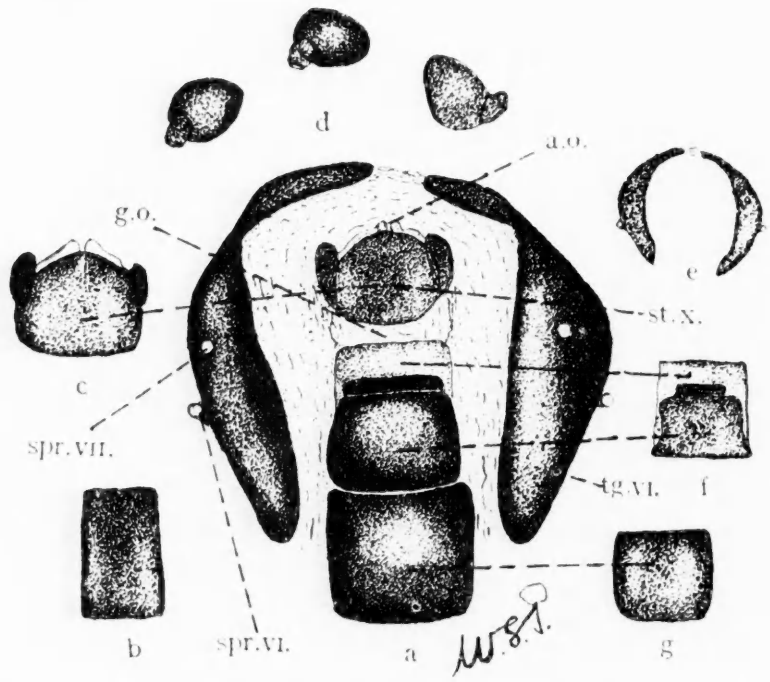

Fig. 9. *a*.—Ventral view of terminalia of ♀ *nigriventris* to show diagnostic characters ; lettering as in fig. 2, *a* ; *b*.—Fifth sternum ; *c*.—Tenth sternum and anal cerci ; *d*.—Spermathecae ; *e*.—Sixth tergum to show division into two plates ; *f*.—Seventh, ninth (?) sternum ; *g*.—Sixth sternum British specimen).

♀. The ♀ terminalia of *nigriventris* are illustrated in fig. 9. Here again they should be compared with those of *clathrata*, for which they may easily be mistaken. In both, tergum 6 consists of two distinct plates. In *nigriventris* tergum 7 is wanting, whereas in *clathrata* it consists of two narrow plates. In

both, terga 9 and 10 are wanting. Sternum 7 in *nigriventris* (fig. 9, *f*) has what appears to be two distinct parts at the distal end, one short, dark and sometimes narrower than the rest of sternum 7, and the other—the more distal part—longer and more lightly chitinized. In *clathrata* there is only a large lightly-chitinized plate at the distal end of sternum 7. The signum is well developed and of a characteristic shape in *clathrata* ; in *nigriventris* it is wanting.

Sarcophaga frenata Pandellé. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN TERMINALIA. ♂. This is one of the ' red-tailed ' species, having a conspicuously bright shining red tenth abdominal tergum (the so-called second genital segment), and an equally shining long black seventh tergum (the apparent first genital segment). Having 3 d.c., hind marginals on the second visible abdominal tergum, and a normal row of frontal setae curving outwards at lower end (thus being distinguished from *striata*, which has a perfectly straight row), it has only to be compared with *haemorrhoa* Mg., which is very similar. Normally it should be possible to recognize *haemorrhoa* by a row of setae on the first long wing vein, and *frenata* by the absence of such a row ; but it is a variable character, and *frenata* generally (at any rate in British specimens) has one or two scattered bristles at the base and sometimes a well-developed row, whilst occasionally they fail in *haemorrhoa*. Both species, moreover, are variable in other respects, especially with regard to the fringes of long fine hairs on the antero- and postero-ventral aspects of the hind tibiae, which in robust specimens are usually well developed, though they sometimes fail entirely, especially in small individuals. It is consequently not always easy to separate the two species on external characters alone, although the terminalia provide excellent distinctions, the anal cerci, the distal segments of ninth coxites and the phallosome being very different. The frons is usually wider in *frenata*, about three-fifths of eye-width, and in *haemorrhoa* only about two-fifths ; the frontalia in *frenata* are about double the width of an orbit, and in *haemorrhoa* only a trifle wider ; *frenata* has a costal thorn, *haemorrhoa* none ; there is some silvery tomentum on tergum 7 in *haemorrhoa*, while in *frenata* it is entirely shining ; *haemorrhoa* is a greyer-looking fly, and *frenata* tends to a more blue-black general tone ; and the long seventh tergum is characteristic of *frenata*, and is about half as long again as wide.

S. frenata has short antennae, the third joint about half as long again as the second ; a full row of moderately strong genal setae ; no presutural acrostichals ; no presutural intra-alar ; one pair of moderate prescutellar acrostichals ; a pair of crossed apical scutellars ; a short row of setae on hind margin of tergum 7 ; third and fifth costal sections nearly equal ; sterna with short hairs ; lamellae of sternum 5 with strongly developed brush of setae (fig. 10, *g*) and some long marginal bristles. LEG CHAETOTAXY. Fore femora with usual postero-ventral series complete ; mid femora with antero-ventral row short and scattered in basal portion, forming a fine comb at apex ; postero-ventral series complete, basal section long and moderately strong, apical portion forming a comb-like

row of fine bristles ; hind femora with antero-ventral series irregular and scattered and moderately strong ; post-ventral series complete, with irregular row of long fine bristles.

♀. In this sex it is still more difficult to separate *frenata* from *haemorrhoa*. Normally, again, the presence or absence of setae on the first long vein should serve, but as in the ♂ variation makes the character uncertain. There appears, however, to be a difference in the shape of tergum 6 (the first ring-like red terminal segment), the upper hind margin of which is straight in *haemorrhoa*, but deeply indented in *frenata* ; whether this is a constant and reliable character we are not quite sure, but if so it affords a ready and easy means for distinguishing the two species.

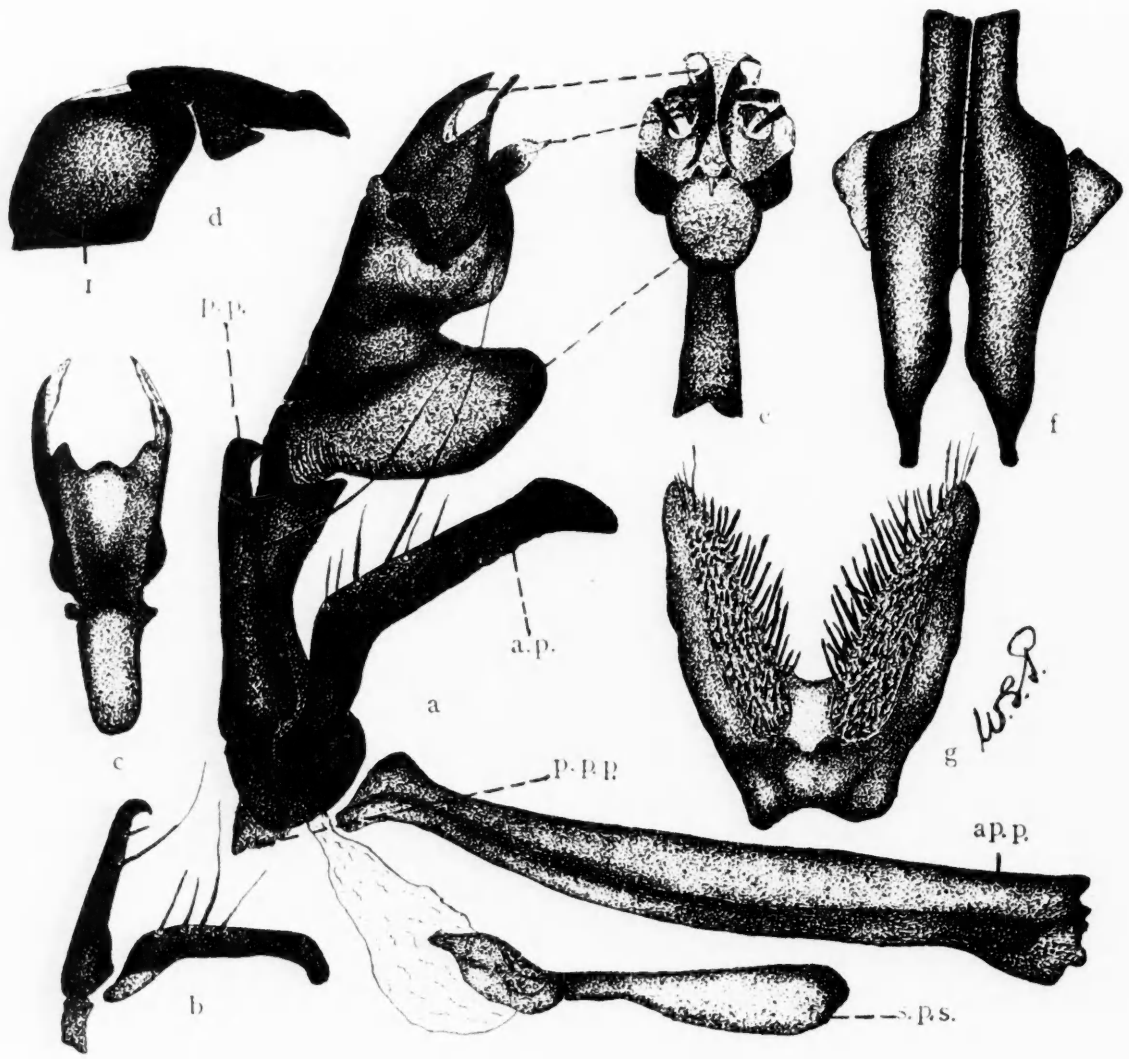

FIG. 10. *a*.—Phallosome and one paramere of *frenata* in side view ; lettering as in fig. 1, *a* ; *b*.—Lateral view of right paramere ; *c*.—Ninth tergo-sternum ; *d*.—Tenth tergum, anal cercus and distal segment of ninth coxite ; note shape of tenth tergum (1) ; *e*.—Dorsal view of phallosome ; *f*.—Ventral view of anal cerci and distal segments of ninth coxites ; *g*.—Fifth sternum (British specimen).

The frons is fully of eye-width; apical scutellars fail; there is a row of strong setae round edge of sixth tergum. The mid femora have fewer long bristles in basal part of both series, but they are long and strong; there is a fine comb-like apical row in both series. The hind femora have some long bristles in basal part of postero-ventral series, with only a slight row of fine short apicals; the antero-ventral series, as in the ♂, irregular and scattered but strong.

NOTES. *S. frenata* is probably the commonest of the 'red-tailed' Sarcophagas in this country; it is widely distributed and we have records or specimens coming from many localities of such varied character as Torquay, Studland, Chippenham Fen, Clayton on South Downs and Wyre Forest, though there are none at present from Scotland or Ireland. It appears to be equally common over much of Europe: Böttcher records it from Germany, Austria, Hungary, France and Italy, and Lundbeck adds Denmark, where, however, it is rare.

Those individuals with a row of setae on the first long vein have been called *cruentata* Pand. and treated as a variety of *frenata*; as already noted, however, we find that British specimens vary indefinitely, and that the character seems of doubtful taxonomic value.

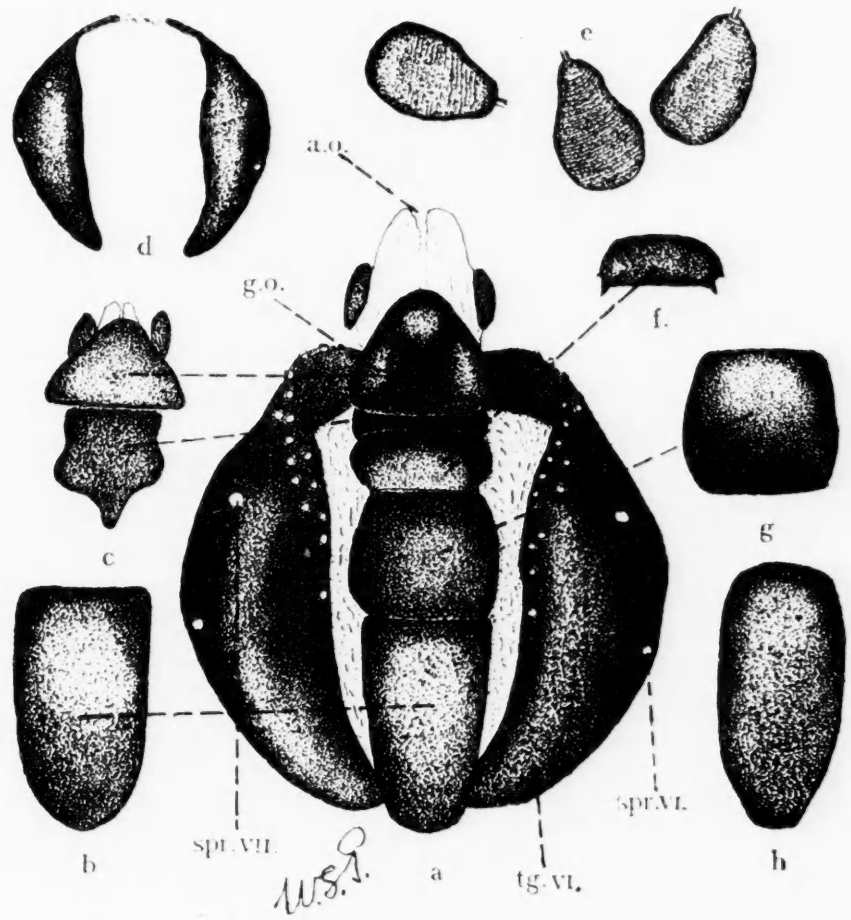

FIG. 11. *a*.—Ventral view of terminalia of ♀ *frenata* to show diagnostic characters; lettering as in fig. 2, *a*; *b*.—Sixth sternum; *c*.—Tenth tergum, anal cerci and accessory plate (?); *d*.—Sixth tergum to show division into two plates; *e*.—Spermathecae; *f*.—Ninth sternum (?); *g*.—Seventh sternum; *h*.—Fifth sternum (British specimen).

Diagnostic Characters of Terminalia. ♂. The ♂ terminalia of *frenata* are illustrated in fig. 10. In this species the terminalia are reddish, and tergum 10 is a rather long plate (fig. 10, *d*). The anal cerci (fig. 10, *d*, *f*) have a characteristic appearance in side view (fig. 10, *d*, which is drawn to a much smaller scale than fig. 10, *f*), the end is raised, terminating in a beak-like point with a very slight notch. Sternum 5 (fig. 10, *g*) has long wide processes, covered with short and long blunt stiff hairs. The phallosome (fig. 10, *a*) has a characteristic appearance in side view. The proximal part is a long, narrow, chitinous tube, and the membrane attaching it to the distal part projects dorsally as a lobe. At the distal end there are several processes, as shown in the illustration. The paramere, too, is characteristic ; the posterior part is a long stout plate ending abruptly in a short beak-like point ; the anterior part is long, bent and broad at the distal end.

♀. The ♀ terminalia are illustrated in fig. 11. Tergum 6 consists of two distinct plates. Terga 7, 9 and 10 are wanting. Sternum 7 has a very distinct plate attached to its distal end, which appears to be sternum 9, and sternum 10 has a large pointed plate (fig. 11, *c*) attached to its lower part (like that in the case of *dissimilis*), which forms the posterior wall of the genital opening. The signum is wanting in this species.

(*To be continued*)

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